Anti-Cardiolipin Antibodies and Recurrent Early Pregnancy Loss

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ABSTRACT

Anti-phospholipid syndrome (APS), first proposed as a distinct disorder in 1983, is a heterogeneous condition requiring fulfillment of both clinical (vascular thrombosis and/or adverse pregnancy outcome) and laboratory criteria (confirmed presence of an anti-phospholipid antibody, aPL) for classification.

In the quarter century since its first description, clinical conditions associated with APS, in addition to systemic lupus erythematosus (SLE), have expanded to include among others, epilepsy, transverse, myelopathy, Guillain-Barré syndrome, Addison's disease, and autoimmune hemolytic anemia. Within each condition, the proportion of patients positive for aPL has been shown to have a less favourable prognosis. It has been observed, for example, that when aPL are present in patients with SLE, there is an increased likelihood of an exacerbation of symptoms. However, APS, particularly when aPL are present with early recurrent pregnancy loss alone rather than with thrombotic manifestations, often has no longterm sequelae.

While acknowledging incomplete understanding of the pathogenesis of tissue injury and fetal loss in APS, investigators have proposed diverse potential mechanisms of complement-mediated inflammation involving platelet and endothelial cell activation using murine models as well as procoagulant effects of aPL acting directly on clotting pathway components. Despite these hypotheses, however, and although aPL remain an essential element in the APS classification criteria, evidence of their pathogenicity in humans remains unconfirmed. Many patients with a long history of thrombotic events or recurrent pregnancy loss, in the absence of comorbidities, are either negative for or have fluctuating levels of aPL. Others repeatedly positive for aPL never experience any of the clinical conditions included in

the classification criteria for APS. Evidently, therefore, the presence of aPL is neither necessary nor sufficient for the development of either vascular thrombosis or recurrent pregnancy loss.

Several randomized clinical trials evaluating therapeutic efficacy for recurrent pregnancy loss have found that live birth rates are not significantly different regardless of treatment or presence or absence of aPL. In addition, longterm studies have shown that the vast majority of aPL-positive women with recurrent pregnancy loss will not have any subsequent thrombotic sequelae or develop autoimmune disease.

On the basis of both clinical and laboratory experience over a 20-year period, it does not seem reasonable to include this group of women, whose only clinical manifestation of APS is recurrent early pregnancy loss, under the same umbrella syndrome as those with catastrophic or recurrent venous or arterial thrombosis, the subsequent requirement for lifelong anticoagulation, and the longterm emotional and financial burden of diagnosis with an uninsurable condition. Two options are therefore proposed to accommodate these findings: either (1) the creation of a new subset of transient APS to include clinical and laboratory classification criteria for women with recurrent pregnancy loss that more appropriately reflects the limited nature and more positive prognosis of their clinical condition or (2) the removal of early recurrent pregnancy loss altogether from the classification criteria of APS. The transient nature of the symptoms and the weak association if any of aPL with the clinical condition indicate the need for this new paradigm for APS.

Introduction

This context statement describes research concerning the prevalence of antiphospholipid antibodies (aPL) in patients with recurrent pregnancy loss, whether with antiphospholipid syndrome (APS), with systemic lupus erythematosus (SLE), or with no co-morbidity. APS, first proposed as a distinct pathological disorder in 1983, is a heterogeneous condition that requires fulfillment of both clinical and laboratory criteria for classification. Definition of the measures by which those criteria are met has been the focus of many studies over the intervening 20 years. Lack of standardization of both laboratory and clinical parameters continues to be a source of contention and has lead to potential over-diagnosis of this condition and confusion surrounding its associated symptoms and sequelae.

My research has involved the development of assay systems for the detection of various aPL and their distribution among women with a variety of clinical conditions. Presence of aPL has been an inclusion criterion for prospective, controlled, randomized trials as well as for many observational, retrospective, and longterm followup trials. These studies were designed to measure treatment efficacy or observe the correlation between presence of aPL and adverse obstetric outcome and the results have been consistent. However, the findings from these many studies do not support the contention that aPL are related in a causative manner to the clinical criteria, particularly to early recurrent pregnancy loss, in APS.

The works highlighted cited in this context statement (including original research papers, editorials, reviews, book chapters and abstracts presented at national and international meetings) had a team of authors and my personal contribution to each requires explanation. I conceptualized and designed 5 of the

studies, performed the laboratory investigations, collection and collation of data, statistical analyses, data interpretation and principal authorship of the manuscripts (Soloninka *et al*, 1995; Clark *et al*, 2002, 2003, 2004, 2005). I was a co-author of 2 editorials that first raised and later reiterated the controversial nature of anti-cardiolipin antibodies and recurrent pregnancy loss (Laskin and Soloninka, 1988; Clark, *et al*, 2001). For 2 randomized, controlled clinical trials (Laskin *et al*, 1997, 2009) I performed the immunological testing and contributed to the interpretation of data originally collated by the study's data management firm. I was a principal co-author of both papers, not only co-writing the submitted manuscript, but also editing the final manuscript to address reviewers' concerns. I performed the laboratory analysis, collated and analyzed the data, interpreted the results and either wrote the manuscripts or prepared the presentations for most of the conference abstracts. Co-author statements of my individual contribution to the research are provided in Appendix 2.

This context statement begins with a review of the APS literature over the past 25 years presented in a chronological manner. Points of contention that have arisen and attempts to reach consensus are reviewed. A brief summary of unresolved issues and a proposal for their resolution follows the literature review. The works cited in support of this proposal are presented in Chapters 3 and 4, divided into serological and clinical contributions to knowledge. The context statement concludes with a discussion (Chapter 5) that synthesizes the findings into concise points.

Chapter 1. Literature Review

Systemic lupus erythematosus (SLE) is a non-organ specific, multi-systemic autoimmune disease of unknown etiology with numerous immunological and clinical manifestations. Frequent signs and symptoms of SLE include photosensitivity, arthritis, leucopenia and glomerulonephritis, usually accompanied by circulating autoantibodies. The increased frequency of women with SLE is attributed to an estrogen hormonal effect which accounts for the differing female-to-male ratio in various age groups: 3:1 in children, between 7-15:1 in adults, and 8:1 in older patients[1]. SLE has traditionally been described as a condition associated with poor pregnancy outcome, although Improvements in disease management and perinatal monitoring have resulted in a significant decrease in pregnancy loss in SLE over the last 40 years and a trend toward decreased preterm deliveries over the last 20 years[2].

Historical Perspective

In the early 1950s, it was noted that about 10-20% of patients with SLE had a biological false positive result for syphilis (BFP-STS)[3]. For about 20 years, a chronic BFP-STS (lasting longer than 6 months) was included in the serological diagnostic criteria for SLE. It was determined in the 1980s by Harris *et al*, that one of the constituent phospholipids used in the syphilis test, cardiolipin, a negatively charged molecule, was the antigen responsible for the false positive results frequently seen in patients with SLE[4]. Subsequent development of a radioimmunoassay, and later an enzyme-linked immunosorbent assay (ELISA) enabled specific detection of anticardiolipin antibodies. In 1983, Hughes *et al* proposed a novel, pathological

disorder, distinct from SLE, the anti-phospholipid syndrome (APS)[5].

Classification Criteria for the Antiphospholipid Syndrome

APS has evolved over 25 years into a condition with a diverse spectrum of clinical associations but when first described, there was a limited set of clinical and laboratory classification criteria. These have been modified somewhat over the years (Sapporo Criteria, 1999[6]; Sydney Criteria, 2006[7], Table 1), but still require evidence of both one or more specific, documented clinical events *and* the confirmed presence of an antiphospholipid antibody (aPL). Clinical criteria comprise either an episode of vascular thrombosis and/or adverse pregnancy outcome including late pregnancy loss or recurrent early pregnancy loss. The laboratory criteria initially required the presence of either a lupus anticoagulant and/or anticardiolipin antibodies on at least 2 occasions not less than 6 weeks apart. This has been subsequently modified to include any one or more of the following: presence of anti-β2 glycoprotein IgG or IgM antibodies (β2GPI), moderate to high titre anticardiolipin IgG or IgM, and/or the lupus anticoagulant, demonstrated on at least 2 occasions, not less than 12 weeks apart. The initial Sapporo classification criteria for APS (Table 1) were replaced in 2006 by the Sydney criteria (Table 1), each representative of the location of the meeting at which the criteria were defined. The clinical obstetric portion of the Sydney criteria expanded on Sapporo criteria to include placental insufficiency as a classification criterion in addition to eclampsia and pre-eclampsia.

There are 3 distinct APS disease entities: primary (the absence of any comorbidity), secondary (when there is a pre-existing autoimmune condition, most frequently SLE), and catastrophic (when there is simultaneous multi-organ failure with small vessel occlusion).

Table 1. Comparison of Sapporo[6] and Sydney[7] classification criteria for the antiphospholipid syndrome (APS).

Criteria	Sapporo (1999)	Sydney (2006)
Clinical		
Vascular thrombosis		
Event	≥ 1 clinical episode of arterial, venous or small-vessel thrombosis in any tissue or organ	≥ 1 documented episode of arterial, venous, or small vessel thrombosis—other than superficial venous thrombosis—in any tissue or organ
Confirmation	By imaging or Doppler studies or histopathology	By objective validated criteria.
Exception	No significant inflammation in the vessel wall	No significant evidence of inflammation in the vessel wall
Adverse pregnancy outcome		
Pregnancy mortality	≥ 1 unexplained death of a morphologically normal fetus (documented by ultrasound or direct examination of fetus) at or beyond the 10th week of gestation or	≥ 1 unexplained death of a morphologically normal fetus (documented by ultrasound or direct examination of the fetus) at or beyond the 10th week of gestation or
	≥ 3 unexplained consecutive spontaneous abortions before the 10th week of gestation with maternal anatomic or hormonal abnormalities, and paternal and maternal chromosomal abnormalities excluded	≥ 3 unexplained consecutive spontaneous abortions before the 10th week of gestation, with maternal anatomic or hormonal abnormalities and paternal and maternal chromosomal causes excluded
Pregnancy morbidity	≥ 1 premature birth of a morphologically normal neonate at or before the 34th week of gestation due to severe pre-eclampsia or eclampsia	≥ 1 premature birth of a morphologically normal neonate before the 34th week of gestation due to eclampsia or severe pre-eclampsia according to standard definitions, or recognized features of placental insufficiency,
Laboratory		
Anti-cardiolipin IgG and/or IgM	Measured by standardized cofactor-dependant ELISA; On 2 or more occasions ≥ 6 weeks apart; Medium or high titre (not specified)	Measured by standardized, non-cofactor dependent ELISA; On 2 or more occasions, ≥ 12 weeks apart ; Medium or high titre (i.e., > 40 GPL or MPL, or > the 99th percentile),
Lupus anticoagulant	Detected on 2 occasions ≥ 6 weeks apart according to the guidelines of the International Society of Thrombosis and Hemostasis	Detected on 2 occasions ≥ 12 weeks apart according to the guidelines of the International Society of Thrombosis and Hemostasis
Anti-β2 glycoprotein I IgG and/or IgM	Not included	Measured by standardized ELISA; On 2 or more occasions, ≥ 12 weeks apart; Titre > the 99th percentile

Etiology of APS

The mechanism(s) causing the production of aPL remain unknown. However, like other autoimmune diseases, APS is considered to derive from a combination of environmental (infectious agents, trauma, and drugs) and genetic factors. In 1998, Gharavi and colleagues hypothesized that viral infection was capable of inducing "pathogenic" aPL. They reported that mice immunized with foreign β2GP1 developed spinal cord infarction that resulted in intrauterine fetal death and transverse myelopathy[8]. It was noted by a number of investigators that the detection of aPL were associated in humans with a number of viral infections including but not limited to hepatitis C, human immunodeficiency virus, cytomegalovirus, varicella zoster, Epstein-Barr virus, adenovirus, and parvovirus B19[9]. Five years later it was hypothesized after further studies with cytomegalovirus in mice that a limited number of aPL induced by various bacterial and viral products would be pathogenic in predisposed individuals. It was postulated that identification of these bacterial and/or viral agents might indicate strategies for the prevention of production of pathogenic aPL or that they might be useful for the induction of tolerance against aPL[10].

Other investigators have proposed molecular mimicry as one aspect of the multifactoral etiologies of APS. Harel *et al* reviewed the animal models that have shown a link between infection and APS[11]. Mice immunized with various pathogenic agents have developed clinical or laboratory evidence of APS-like disease. It has been established that epitopes of β2GP1 share similarities with common infectious pathogens and anti-β2GP1 titres were found to be particularly high after mice were injected with *Haemophilus influenzae*, *Neisseria gonorrheae* in addition to

immunogens such as tetanus toxoid.

It is evident that although aPL have been associated with many infections in humans, only in a few cases do these antibodies have any pathogenic potential. In fact anticardiolipin antibodies have become referred to as either "infectious" (usually the IgM isotype) or "autoimmune"[12] although this distinction may not be absolute. The debate regarding the pathogenesis of aPL (and the evolution, therefore, of APS) is a continuing one, with some indicating their support for the concept that aPL initially play a key and beneficial role in the innate immune response and are only pathogenic among genetically susceptible individuals as a result of adverse intravascular events[13]. Others feel that aPL, produced perhaps as a result of molecular mimicry, are immediately pathogenic because in about one third of cases, there is a clear association with a predisposing infection immediately prior to the onset of catastrophic APS[14].

Molecular Pathogenesis of aPL

Early concepts in the pathogenesis of aPL included the possibility that aPL inhibits prostacyclin generation by epithelial cells or that they had procoagulant activity[15,16]. It was hypothesized that aPL affected the balance between the procoagulant and anticoagulant states by interacting with the protein C system and prothrombin for example, resulting in thrombin generation[17]. Using an animal model of thrombosis, Pierangeli *et al* showed that human polyclonal and monoclonal aPL, derived from a patient with APS, reversibly enhanced thrombus formation in mice[18]. However, it was apparent that there was no single mechanism linking the presence of aPL and the development of thrombosis despite numerous investigations (Table 2)[15-19]. Rand[19]

postulated 3 possible scenarios: that (1) the primary pathogenic process might be the exposure of highly thrombogenic anionic phospholipids through some other process and that aPL could be the result of an autoimmune response to those anionic phospholipids in susceptible individuals; that (2) aPL might both cause and react to the exposure of the anionic phospholipids by initiating a thrombotic event thus exposing anionic phospholipids which in turn provoke an autoimmune response resulting in the production of more aPL which in susceptible hosts have additional thrombogenic properties and promote a vicious circle; or finally (3) that aPL may be simply an epiphenomenon or marker not directly involved in cause and effect mechanisms of the pathogenesis of APS. He also presented a new hypothesis involving aPL in the displacement of circulating annexin V, a phospholipid binding protein with thromboregulatory activity.

By 1998, β2GP1 had been identified as the target antigen for aPL and the essential cofactor for binding of anti-cardiolipin antibodies[20]. Still their role in thrombin formation remained unclear although by this time, molecular biologists and pharmaceutical neurologists had joined immunologists, hematologists, rheumatologists and obstetricians in attempting to understand the pathology of APS[20]. A number of potential thrombogenic roles for aPL were proposed in addition to the annexin V theory including inhibition of fibrinolytic and activated protein C activity[21,22] and binding to antigens on endothelial and trophoblast cell surfaces[23]. Although some provided insights into the relationship between aPL and fetal loss,

Table 2. Mechanisms proposed for the pathogenesis of APS. (Reproduced from *Rand JH. Molecular pathogenesis of the antiphospholipid syndrome. Circ Res 2002;90:29-37* by kind permission; license # 229311060531.)

Interference with a phospholipid- or other polyanionic-dependent antithrombotic mechanism
Exposure of anionic phospholipid through disruption of the annexin-V shield
Interferences with protein C pathway
aPL binding to proteins C and S
Inhibition of activation of protein C
Acquired activated protein C resistance
Inhibition of tissue factor pathway inhibitor
Impairment of phospholipid-mediated autoactivation of factor XII and reduced fibrinolysis
Inhibition of heparin-antithrombin-III complexes
Promotion of tissue factor exposure/synthesis
Induction of tissue factor expression on monocytes and on endothelial cells
Vascular injury/stimulation of apoptosis
Injury to endothelium
Induction of apoptosis on vascular cells
Release of membrane-bound microparticles
Promotion of cellular adhesion to vascular surfaces
Induction of receptors for cell adhesion molecules on endothelium
Stimulation of platelet function
Platelet activation
Release of membrane-bound microparticles
Others
Antibody-mediated alteration of eicosanoid synthesis
Increase of endothelin-1
Cross-reactivity to oxidized LDL
Increase of plasminogen activator inhibitor-I

very few satisfactorily explained the link with thrombosis[24].

In 2004, Salmon and Girardi proposed complement activation as a central mechanism in aPL-induced pregnancy loss and placental infarction based upon observations after passive transfer of human aPL into pregnant mice[25]. They acknowledged that the cause of tissue damage due to aPL was likely multifactoral, but proposed complement activation as an absolute requirement for the most serious phenotypic outcomes. However, others reported that placental infarction was conspicuously absent from the placentas of aPL-infused mice, and that anticoagulation therapy with heparinoids or the selective thrombin inhibitor hirudin did not prevent fetal loss in mice[26]. Indeed, Girardi *et al* also reported that heparin was effective in preventing aPL-induced fetal loss because of its inhibition of complement activity, not because of its anticoagulant activity[27] in mice.

Despite a number of putative mechanisms proposed (Table 2), the intervening 5 years have not provided any additional significant insights and the collective data still do not show any conclusive evidence for a direct causal relationship between aPL and thrombosis or pregnancy losses in humans. In agreement with Weiler's comments in 2008[26], Rand in 1998[19] and 2002[28], and Shoenfeld in 2003[29] discussed the possibility that aPL may be more of a complicating epiphenomenon rather than the causative pathogenic mechanism of the disease process in APS. Others have reported that there is evidence that aPL may be necessary but are not sufficient to trigger some of the manifestations of the syndrome. The most recent proposition is that "second hits", including infectious agents, smoking, oral contraception, diet and lifestyle, are the necessary triggers for the production not only of cross-reacting aPL

but also of an inflammatory response that potentiates thrombogenesis in genetically susceptible individuals[30,31].

Laboratory Evaluation of APS

In 1985, shortly after APS was first described, the identification of anticardiolipin antibodies by radioimmunoassay appeared to be reliable, sensitive and specific[5]. Solid phase ELISAs were developed for both IgG and IgM isotypes , as well as functional assays for the detection of the lupus anticoagulant[32]. By the early 1990s, it had become apparent that there were a considerable number of issues to be resolved concerning the detection of aPL from the most basic methodology (correct selection of microtitre plate to ensure appropriate epitope presentation[33]) to the identification of a plasma or serum cofactor (β2GP1) necessary for anticardiolipin binding to occur[34,35]. In addition to the methodological issues, standardization of anticardiolipin and lupus anticoagulant detection and the variable sensitivity and specificity of modes of detection both within and between laboratories, became a focal point of aPL investigation[35-37].

There are still controversial issues surrounding methodology for aPL measurement although there has been clarification of some points: IgA isotypes of both anticardiolipin and anti-β2GP1 antibodies have been largely dismissed as insignificant with regards to APS[38]. It is well established that co-factor (β2-GPI)-independent IgM anticardiolipin antibodies are frequently detectable after a variety of viral infections[39,40] so their presence when screening potential patients for APS must be regarded with a degree of skepticism. The updated Sydney classification criteria

for APS attempted to account for the infection-related spike in anticardiolipin IgM production by requiring a repeated measurement not less than 12 weeks after the first positive test rather than the initial 6 week provision for confirmation (Table 1, p.10). The new criteria also included anti-β2GP1 IgG and IgM antibodies although their utility in classifying patients with APS has yet to be fully accepted[41-43]. However, the new criteria dropped the requirement for anticardiolipin antibodies to be measured using a β2GPI-dependent ELISA, thereby greatly reducing the specificity of the assay and enabling the potential misclassification with APS of patients with infection-related (non-β2GP1-dependant) anticardiolipin antibodies[44] .

With regard to assay standardization, the majority of laboratories use commercial kits to detect the presence of anticardiolipin antibodies, and there is a continuing lack of consensus regarding the source, integrity, and necessity of the β2GP1 cofactor, the use of monoclonal antibodies as reference calibrators, and the use of recommended cut-off values by manufacturers compared to those derived by each independent laboratory[45]. These issues of standardization culminated in 2008 with publication of representative papers entitled "Is standardization an impossible dream?"[41], "A quarter of a century in anticardiolipin testing and attempted standardization has led us to here, which is?"[42] and "A potpourri of problems, a compilation of possible solutions"[45]. Because of a dearth of good quality published evidence, existing guidelines were formulated based upon the expert (and therefore inherently subjective) recommendations and personal preferences of the most influential investigators in the field, not by a consensus approach. The guidelines have, as a result, been criticized for being "eminence rather than evidence-based"[46], which has limited the clinical utility of aPL testing and unfortunately undermines the

validity of the laboratory portion of the classification criteria.

Obstetric APS

Adverse pregnancy outcome is one of the clinical classification criteria for APS (Table 1): ≥ 3 consecutive early losses (< 10 weeks' gestation); ≥ one loss at or after 10 weeks' gestation; one or more preterm deliveries (before 34 weeks' gestation) accompanied by eclampsia or pre-eclampsia; evidence of placental insufficiency, usually recognized by intrauterine growth restriction (IUGR) resulting in a fetus with low weight for its gestational age. There have been numerous studies reporting the presence of aPL in recurrent pregnancy loss (RPL)[47-51] . There is no consensus regarding the prevalence of aPL associated with this condition. This may be due to the lack of assay standardization described above in addition to the considerable variation in patients selected for pregnancy-loss treatment trials: some trials have included women with both early and late losses, some specifically excluded women with a history of thrombosis, and some accepted women with anticardiolipin IgM antibodies but excluded those with the lupus anticoagulant[47,52].

Three early trials evaluated the treatment efficacy of immunosuppression, specifically prednisone, and aspirin for recurrent pregnancy loss with aPL[53-55]. It was concluded that prednisone did not confer additional benefit over aspirin alone and that it may have been associated with the higher rate of premature births seen in each trial. As a result, treatment with prednisone for recurrent pregnancy loss has become contraindicated. Subsequently, a number of randomized controlled trials evaluated the efficacy of either unfractionated or low molecular weight heparin for

patients with recurrent pregnancy loss and aPL. Each established its own aPL inclusion criteria (which aPL to measure and cutoffs for each) and intuitively, these study design variations might be expected to have resulted in differential outcomes. However, the live birth rates, whether in the unfractionated or low molecular weight heparin treatment groups, and regardless of the aPL status, are similar, ranging from 71.1% to 80%, with a weighted mean of 77.0%. Despite the observation that specificity and titre of aPL appears to be unrelated to treatment effect, a regimen of low molecular weight heparin and aspirin throughout pregnancy for aPL-positive women with a history of recurrent pregnancy loss has become entrenched[62].

It is interesting to note that some investigators consider the association of aPL and recurrent pregnancy loss historically well-established[62] perhaps because it was initially observed in connection with the high rates of biologically false positive syphilis tests and increased rates of fetal loss in patients with SLE in the 1950s. Technical advances enabled the development of assays for specific phospholipids including the lupus anticoagulant and anti-β2GP1 and anticardiolipin antibodies. In women with otherwise unexplained recurrent pregnancy loss, approximately 15-20% have an aPL[63,64]. A meta analysis in 2006[65] observed that the magnitude of association between aPL and recurrent pregnancy loss in women without SLE varies and depends upon type of aPL measured. Even among the studies included in the meta analysis (25 of an initially retrieved 128 published studies), the authors noted poor standardization of assays, inclusion of women with other causes of recurrent pregnancy loss, inconsistent selection of controls, variability of aPL antibody or isotype tested, and variable definition of recurrent pregnancy loss, (including number and timing of losses). No association was found between pregnancy loss and anti-

β2GP1 antibodies, while there was an association with the lupus anticoagulant and recurrent pregnancy loss. The association between recurrent pregnancy loss and anticardiolipin IgG and IgM anticardiolipin antibodies appeared variable and depended upon the titre and timing of loss. The authors reiterated that prospective human data on the relationship between aPL and recurrent pregnancy loss are still lacking and that the pathophysiology of their role in uteroplacental insufficiency remains incompletely understood.

Longterm Prognosis

Vascular thrombosis and either late or recurrent early pregnancy loss constitute the clinical criteria for APS. Pregnancy loss is necessarily limited to women of child-bearing age, and it has been a debate regarding the longterm sequelae of recurrent losses: do the losses presage a lifelong coagulation disorder manifested by thrombotic episodes, does APS evolve into SLE, or are the pregnancy losses the only clinical manifestation of APS the women will experience regardless of their aPL status? While there are a number of studies that have assessed the need for longterm anticoagulation for patients who initially present with thrombosis[66], it was noted in a 5-year followup of 1000 patients with APS that no clinical or immunologic predictor of thrombotic events, pregnancy morbidity or mortality was detectable[67]. With regards to whether APS evolves in SLE, one study followed 128 patients with APS over a mean of 9 years and confirmed that progression from primary APS to SLE or lupus-like disease is unusual, even after a long followup[68]. A review of the literature reported that between 4-10% of cases of primary APS developed into complete SLE. A longterm followup study of 98 asymptomatic individuals with aPL

found no increased incidence of thrombosis, even in a placebo-treated group[69].

Five studies have reported on thrombosis rates after initial presentation with recurrent pregnancy loss. The earliest study (1994)[70] reported that 34/150 women had a thrombotic event within 3 years: 8 occurred in association with pregnancy, and 8 occurred while the patient was receiving anticoagulant therapy. A second study of 65 patients followed over a mean of 8 years also reported a high rate of thrombosis in APS patients who presented only with pregnancy loss[71]. However others reported that they had not found a high incidence rate (2 patients with strokes, both taking aspirin at the time) in a 5-year followup study of 59 patients in contrast to the earlier findings[72]. Two studies, one in 2005 following 141 women with a history of recurrent pregnancy loss over a mean of 7.2 years[73] and another following 52 women with the same history over a mean of 17 years[74] found low incidence of thrombosis regardless of whether or not they were positive for aPL. It is evident from these disparate findings that the incidence of thrombosis subsequent to recurrent pregnancy loss is controversial at best, appears unrelated to the presence of aPL, is independent of concurrent anticoagulation therapy, and unpredictable as no one has yet been able to identify any clinical or immunologic risk factors.

Chapter 2. Issues and Proposal

Controversy

Upon reviewing the literature from the past 25 years, it is apparent that the classification of APS, its etiology, pathogenesis, and prognosis all remain somewhat undefined. Thousands of papers have been published in this area and yet many aspects describing this syndrome seem to defy clarification. For many of the issues, there appear to be numerous papers that either support or dispute earlier findings regarding presence or absence of aPL-associated cause and clinical effect and treatment efficacy but no conclusive proof. This is especially true with regards to APS-related pregnancy morbidity.

While acknowledging incomplete understanding of the pathogenesis of tissue injury and fetal loss in APS, some investigators, using murine models, have proposed a mechanism of complement-mediated inflammation involving platelet and endothelial cell activation while others have postulated procoagulant effects of aPL acting directly on clotting pathway components[15-23]. Despite these hypotheses, however, and although aPL are an essential element of the classification criteria, evidence in support of their pathogenicity in humans remains equivocal.

The well-described hypercoagulability of pregnancy may well account for the observed increase in antenatal and postnatal thrombosis in pregnancy and may be distinct from the prevalence of aPL that may indeed be simply an epiphenomenon[26]. This hypercoagulability probably evolved to prevent life-threatening loss of blood either in the event of a miscarriage or during labour and delivery.

A number of randomized clinical trials have found no benefit with anticoagulant therapy or any variation in treatment efficacy correlated to variable aPL types and titres (Table 3). There appears to be no increased rate of post-partum thrombosis when comparing women with and without aPL[75]. In addition, longterm followup studies[68-70] have shown that the vast majority of women with recurrent pregnancy loss and aPL will not have any subsequent thrombotic sequelae or develop autoimmune disease[72-75] even as long as 20 years after initial presentation[74].

It has been observed that when aPL are present, there is an increased likelihood of an exacerbation of symptoms of SLE[77-79] including an increased risk of thrombosis and recurrent pregnancy loss. However, in contrast, and importantly, patients with primary APS (with no underlying SLE), particularly when aPL are present only with early recurrent pregnancy loss and not thrombotic manifestations, often have a more benign prognosis and no apparent longterm sequelae[72-74,].

Standard of practice for women with obstetric manifestations of APS (ie recurrent early loss, still birth, or premature delivery of an infant with evidence of placental insufficiency manifested by intrauterine growth restriction) usually receive anticoagulation during pregnancy and immediately post partum because the risk of thrombosis is naturally higher immediately after delivery[80]. If a thromboembolic event occurs, anticoagulation may be continued indefinitely. The treatment regimen does not differ for patients with *recurrent* thromboembolism regardless of the presence or absence of aPL[66,75,76,80,81].

Table 3. Comparison of live birth rates in studies with either unfractionated or low molecular weight heparin arms. Anticardiolipin IgG and IgM levels are reported as GPL or MPL units respectively[61]. ASA: aspirin; aPL: antiphospholipid antibody; aCL: anticardiolipin; LAC: lupus anticoagulant; aPS: anti-phosphatidylserine; sc: subcutaneous; UFH: unfractionated heparin; LMWH: low molecular weight heparin; MOM: multiples of the mean; DRVVT: dilute Russell's viper venom time; PTT-LA: lupus anticoagulant-sensitive partial thromboplastin time; DilPT: dilute prothrombin time; KCT: kaotln clotting time.

Author, Year	n	Determination of aPL Positivity	Intervention	% Live Births
Kutteh, 1996[57]	25	aCL IgG ≥ 27 and/or IgM ≥ 27 (Pos LAC excluded)	ASA + sc UFH	80.0
Rai, 1997[56]	45	aCL IgG > 5 and/or IgM > 3 and/or LAC (RVVT)	ASA + sc UFH	71.1
Cowchock, 1992[51]	26	aCL IgG > 30 or IgM > 11 and/or LAC (DRVVT or APPT)	ASA + sc UFH	73.1
Franklin, 2002[59]	25	aCL IgG > 20 and/or IgM > 20 and/or aPS > 3 MOM and/or LAC (DRVVT)	ASA + sc LMWH	76.0
Farquharson, 2002[58]	51	aCL IgG > 9 and/or IgM > 5 and/or LAC (DRVVT)	ASA + sc LMWH	78.4
Noble, 2005[60]	25	aCL IgG > 20 and/or IgM > 20 or aPS > 3 MOM and/or LAC (DRVVT)	ASA + sc LMWH	80.0
Laskin, 2009[61]	22	aCL IgG > 15 and/or IgM > 25 and/or LAC (DRVVT, PTTLA, DilPT, KCT)	ASA + sc LMWH	77.3

Many patients with a long history of thrombotic events or recurrent pregnancy loss, in the absence of comorbidities like SLE, are either negative for or have fluctuating levels of aPL[77]. It was reported that history of atrial fibrillation, congestive heart failure and valvular heart disease was found to be associated with significantly higher levels of anticardiolipin IgG but it was observed that the increases in immunoreactivity were a function, in a dose-dependent manner, of the number of cerebrovascular risk factors also present[82]. The authors therefore cautioned against over-diagnosis with APS and the consequent changes in management among patients with multiple risk factors. In addition, aPL are found in patients with malignancies, in the normal population, and in patients after infections, but without any association with any of the clinical conditions included in the classification criteria for APS[12,83-85]. It has been proposed that the figure of "1 in 5 is a useful guide"[64] : 1 in 5 patients with recurrent pregnancy loss have aPL and 1 in 5 patients with deep vein thrombosis have aPL. The corollary to this is that 4 out of 5 patients with recurrent pregnancy loss do not have aPL; neither do 4 out of 5 patients with deep vein thrombosis. It is evident, therefore, that the presence of aPL is neither necessary nor sufficient for the development of either vascular thrombosis or recurrent pregnancy loss.

Summary of controversial issues:

- The vast majority of women with early recurrent pregnancy loss are negative for aPL.

- The vast majority of patients with early recurrent pregnancy loss who are repeatedly positive for aPL never experience any of the non-pregnancy-related clinical conditions included in the classification criteria for APS.
- aPL are neither necessary nor sufficient for the development of early recurrent pregnancy loss.

Proposal

Classification with APS carries the burden of significant longterm health and financial implications because of (1) the influence of classification on subsequent disease management; (2) because of its designation by insurance companies as an uninsurable condition[86-88] and (3) because once classified with this disorder, there are no mechanisms for reversal of the classification despite the potential for absence of recurrent clinical events in many APS patients later in life. It follows, therefore, that classification criteria should be as specific and sensitive as possible.

As Branch stated in 1998, "It is very unlikely that patients with such disparate clinical and laboratory findings have the same autoimmune syndrome" [52]. After contributing our evidence to the ongoing debate surrounding APS and considering our consistent findings, it is difficult to rationalize the continued inclusion of a group of aPL-positive women, whose only clinical manifestation of APS is recurrent early pregnancy loss, under the same umbrella syndrome as those with catastrophic venous or arterial thrombosis and the subsequent requirement for lifelong anticoagulation. Indeed, as will be shown, the findings from our published works illustrate the transient nature of the clinical manifestations and the weak association, if any, of aPL with recurrent pregnancy loss, indicating the need for a new paradigm for APS. It is proposed,

therefore, that either (1) a new subset of APS should be defined that includes clinical and laboratory classification criteria for women with recurrent pregnancy loss and more appropriately reflects the limited nature and more positive prognosis of their clinical condition or (2) early recurrent pregnancy loss should be withdrawn from the classification criteria of APS.

Chapter 3. Contribution to Knowledge

Serological Studies

Methodology

The first classification criteria for APS[6] specified a requirement for medium or high titre anticardiolipin antibodies measured using a standardized, cofactor-dependant assay on 2 occasions, not less than 6 weeks apart. However, these criteria were not published until 1999, more than 15 years after APS had first been proposed as a recognizable and distinct syndrome. For the first decade or so of research in this area, therefore, the measurement of anticardiolipin antibodies was highly variable in methodology, interpretation, and application of results.

Concerns over this lack of standardization were reported in an editorial [35]. While it was well understood that the lupus anticoagulant was associated with the high rates of fetal loss seen in many patients with SLE, it appeared that an equally strong association was being assumed with regard to anticardiolipin antibodies and recurrent fetal loss, even when not in the context of SLE[89-92]. The prevalence of anticardiolipin antibodies reported in patients with SLE[5] (61%) was much higher than in women with idiopathic recurrent pregnancy losses (11%)[93]. Despite studies that did not find an association[93,94], a significant association between anticardiolipin and recurrent losses was becoming accepted[95-98]. Our editorial proposed caution based on those contradictory reports. We also based our cautionary approach on our own (at that point) unpublished findings that anticardiolipin antibodies were also present in a sample of healthy pregnant patients and on our belief that the disparate results

in the literature were in large part due to variable methodologies for assessing the presence of anticardiolipin antibodies.

In an effort to improve the detection of anticardiolipin antibodies, address concerns regarding methodologies, and clarify their prevalence among women with fetal losses, we developed a novel, specific, enzyme-linked immunosorbent assay replacing the evaporation of soluble cardiolipin as the antigen binding step with cardiolipin micelles prepared by sonication, suspended in physiological saline[37]. We evaluated not only different preparations of cardiolipin, but also the use of different washing solutions (with and without the detergent Tween 20[97]), different enzyme conjugates (urease, horseradish peroxidase, and alkaline phosphatase), and different blocking agents (fetal bovine serum, horse serum and normal human serum). The specificity of the ELISA was determined by pre-incubation of control positive and negative sera not only with serial dilutions of cardiolipin, but also with dilutions of other phospholipids (phosphatidyl serine, phosphatyidylinositol, and phosphatidic acid) as well as irrelevant antigens (double-stranded DNA and ovalbumin)[98,99]. The most sensitive assay system included the use of protamine sulphate-precoated, polystyrene flat-bottom microtitre plates, cardiolipin micelles, horse serum in the diluent, and urease conjugated rabbit- anti-human IgG and IgM.

Distribution of Anticardiolipin Antibodies

Once we had established a reliable and specific assay system, we measured anticardiolipin IgG and IgM in the following groups: non-pregnant controls, healthy pregnant women, and women with a history of recurrent pregnancy loss. The issue

of determining appropriate cut-off levels for an upper limit of normal continues to be controversial with anticardiolipin assays[42]. We supported the early observation that normal values are positively skewed and that ideally, non-parametric analyses should be used to establish confidence intervals. Using ROC (receiver operator characteristics) graphs comparing results from healthy, non-pregnant women and women with a history of recurrent pregnancy loss to determine the cutoff levels for each isotype that minimized false positive and negative results, we identified a titre that distinguished between IgG levels in controls and women with recurrent pregnancy loss, but found that the frequency of anticardiolipin IgM was not significantly different in healthy pregnant women and those with a history of recurrent pregnancy loss. Because of the relative non-specific distribution we observed and its acknowledged association with viral infection, we concluded by cautioning against basing any clinical decisions on the presence of this antibody alone particularly as a criterion for therapeutic intervention. We also investigated the presence of anticardiolipin IgA and found that it was rarely found and of no diagnostic value for women with recurrent pregnancy loss[100,] confirming previous findings[101,102].

Next we evaluated 1185 women for a number of autoantibodies including anticardiolipin IgG[50,51:] : 700 had a history of recurrent fetal loss and were being screened for participation in a randomized controlled clinical trial[55] and 485 were healthy controls. Several different assay systems, including our in-house assay and 2 commercial kits, were used to determine the prevalence of anticardiolipin IgG. We also carried out a detailed literature review to place our findings in context. We found anticardiolipin IgG in 3.9% of women with recurrent loss. We surveyed 185 publications and selected for inclusion only those which reported appropriately

standardized anticardiolipin IgG levels in women with similarly stringently investigated unexplained recurrent pregnancy loss. We identified 14 additional papers resulting in a total sample of 2702 women. The sample sizes in the different studies ranged from 35 to 700 (ours) and 147/2702 (5.4%) were positive for anticardiolipin IgG. These findings supported our earlier observations: that this antibody was not found frequently in this population and therefore probably not strongly associated with recurrent pregnancy loss.

Anticardiolipin Antibodies in Parvovirus B19 Infection

As part of a different research project[103], sera from 91 patients with either acute, convalescent, or no parvovirus B19 infection were assayed for the presence of anticardiolipin IgG[40]. Patients with acute B19 infection were positive for anticardiolipin IgG significantly more frequently than those with either convalescent or no B19 infection. Our findings contributed to the understanding, initially observed with human immunodeficiency virus (HIV)[104] in the late 1980s, that many viral infections are associated with transient anticardiolipin antibody production outside the context of APS[12]. Levels of anti-B19 positivity were not correlated with levels of anticardiolipin IgG, indicating that the anticardiolipin antibodies were not merely a result of polyclonal B-cell activation, but a specific response to parvovirus infection. However, the decrease in anticardiolipin antibody prevalence in convalescent sera supported the contention that they were transient and unrelated to any aPL-related clinical manifestations including thrombosis or recurrent pregnancy loss. This was one of the earliest reports of anticardiolipin antibodies during parvovirus B19 infection.

Serologic Investigation for Recurrent Pregnancy Loss

Women with a history of recurrent pregnancy loss undergo a number of investigations to exclude the following etiological factors for their adverse obstetric outcomes: anatomic (sonohystogram), hormonal (timed endometrial biopsy), and genetic (karyotype analysis of both partners). Those found with an abnormality are referred for appropriate treatment or counselling. The remaining patients are screened, at our clinic, for a number of immunologic and coagulation abnormalities. We evaluated 783 women with otherwise unexplained recurrent pregnancy loss who were being screened for participation in a randomized clinical trial. We found that 21.2% had a lupus anticoagulant (LAC), whereas only 3.9% were positive for anticardiolipin IgG[51], consistent with our earlier observations that the LAC is more prevalent among this population than anticardiolipin antibodies. We also noted that 24% had an autoantibody other than an aPL although none had any clinical manifestation of a connective tissue disease. These results supported the contention that it is not sufficient to screen women with a history of recurrent pregnancy loss only for aPL (anticardiolipin or LAC) as there may be other autoimmune or inflammatory processes in play that may contribute to an adverse obstetric outcome. In addition, it was important to note that more than 50% of women with recurrent pregnancy loss had no detectable anatomic, hormonal, genetic, immunologic or coagulation abnormality and whose losses could therefore be termed idiopathic.

We also investigated the prevalence and specificity of anticardiolipin and anti-β2GP1 antibodies, comparing patients with recurrent pregnancy loss (n = 84), those

classified with APS with pregnancy loss only (n = 30), and those with APS with thrombosis (n = 13). Both anticardiolipin and anti-β2GP1 antibodies were far more frequently found in APS patients with a history of thrombosis than in either of the other patient groups[105], indicating that they are probably better markers of hypercoagulability than of pregnancy loss. The number of patients with a history of thrombosis in this study was small but the comparator groups with a history of recurrent pregnancy loss were much larger, so although the positive association with thrombosis might not have represented more than a trend requiring confirmation with a larger study, the lack of association seen with the pregnancy loss groups (n = 114) was statistically reliable.

A subsequent investigation of 133 consecutive patients with SLE found that the anti-β2GP1 antibodies (measured by a commercial ELISA kit) were frequently present (35-40% of the population was positive for at least one isotype)[43]. However, anti-β2GP1 antibodies did not discriminate between SLE patients with or without APS, or between those with and without a history of either thrombosis or recurrent pregnancy loss. We did find that the combined presence of anticardiolipin antibodies, the LAC and anti-β2GP1 antibodies was strongly associated with a history or thrombosis and/or recurrent pregnancy; however, this was seen in only a small minority of patients (8/133) and the number was too small to reach any statistical significance. We concluded that anti-β2GP1 antibodies do not supercede other established assays in identifying patients with clinical features of either SLE or APS. A review of the literature revealed that secondary diagnoses and laboratory methodologies were too variable to permit valid comparisons of results and we were therefore unable to

draw any conclusions regarding anti-β2GP1 antibodies and clinical features of secondary APS in SLE. Bertolaccini *et al* subsequently confirmed that anti-β2GP1 antibodies did not add to the recognition of APS in SLE[102].

Titres and Upper Limits of Normal

It was becoming increasingly evident by the early 2000s that the association of anticardiolipin antibodies and recurrent pregnancy loss was tenuous especially at low antibody titres. Despite the Sapporo classification criteria for APS[6] specifying moderate to high titres (> 40 GPL or MPL or > 99th percentile), many investigators were including women with much lower levels of anticardiolipin in their trials (Table 3). We decided to investigate the distribution of anticardiolipin IgG titres among patients attending our SLE and recurrent pregnancy loss clinics. Instead of selecting patients on the basis of their diagnosis and observing their anticardiolipin titres, we selected 280 consecutive sera from our serum bank: 140 positive for anticardiolipin IgG (> 15.0 GPL, IgG anticardiolipin units) and 140 negative (≤ 15.0 GPL) and then retrospectively reviewed the patients' charts to determine their respective diagnoses[106]. Sera were distributed among the diagnostic groups as follows: recurrent pregnancy loss only: 144/280; SLE: 83/280; and APS (defined for the purposes of this study as having a history of thrombosis to differentiate them from women with obstetric manifestations only): 53/280 (Table 4). There were no women in the recurrent pregnancy loss group with anticardiolipin levels above 32 GPL. Of the 42 (29.9%) women who were positive, 85.7% had levels below 20 GPL. In comparison, 48/53 (90.6%) of APS patients and 50/83 (60.2%) of SLE patients were

Table 4. Distribution of anticardiolipin titres among women with recurrent pregnancy loss (RPL), those classified with systemic lupus erythematosus (SLE), and patients with antiphospholipid syndrome (APS) diagnosed by a clinical history of thrombosis[106]. None of the patients with RPL had an aCL IgG level > 32 GPL in contrast to 45.3% of patients with APS.

Anticardiolipin IgG GPL units	RPL n = 144 (%)	SLE n = 83 (%)	APS n = 53 (%)
≤ 15.0 (negative)	102 (70.8)	33 (39.8)	5 (9.4)
> 15.0 (positive)	42 (29.2)	50 (60.2)	48 (90.6)
16-20	36 (25.0)	20 (24.1)	13 (24.5)
> 32	0	12 (14.4)	24 (45.3)

positive. Titres were significantly higher in APS patients compared to both recurrent pregnancy loss and SLE patients ($p < 0.001$ and $p = 0.04$ respectively).

At that time (2004) the APS classification criteria[6] did not quantitatively define what constituted a "moderate" or "high" value for anticardiolipin antibodies. However, by 2006, the updated criteria[7] specified 40 GPL and MPL (or the 99th percentile) as the lower limits of a moderate result, a value with which we concur as we found that at 32 GPL, anticardiolipin antibodies as measured in our laboratory had a positive predictive value of 96.2% for APS (with thrombosis) versus recurrent pregnancy loss. Prior to this update, women with 3 or more early pregnancy losses (but no history of thrombosis) and an anticardiolipin level lower than 40 GPL, fulfilled the earlier inclusion criteria, and were therefore classified with APS. We found few of our recurrent pregnancy loss patients to be positive for anticardiolipin IgG, and those who were positive had low titres. We felt, therefore, that APS classification criteria needed to be modified to exclude these women from the diagnosis. The results of our studies indicated that this group of women appeared to comprise a subset within APS with a distinct anticardiolipin IgG profile, and that their condition carried a different prognosis requiring a modified therapeutic response compared to APS patients with a history of thrombosis[106].

Anticardiolipin Antibodies and the Lupus Anticoagulant

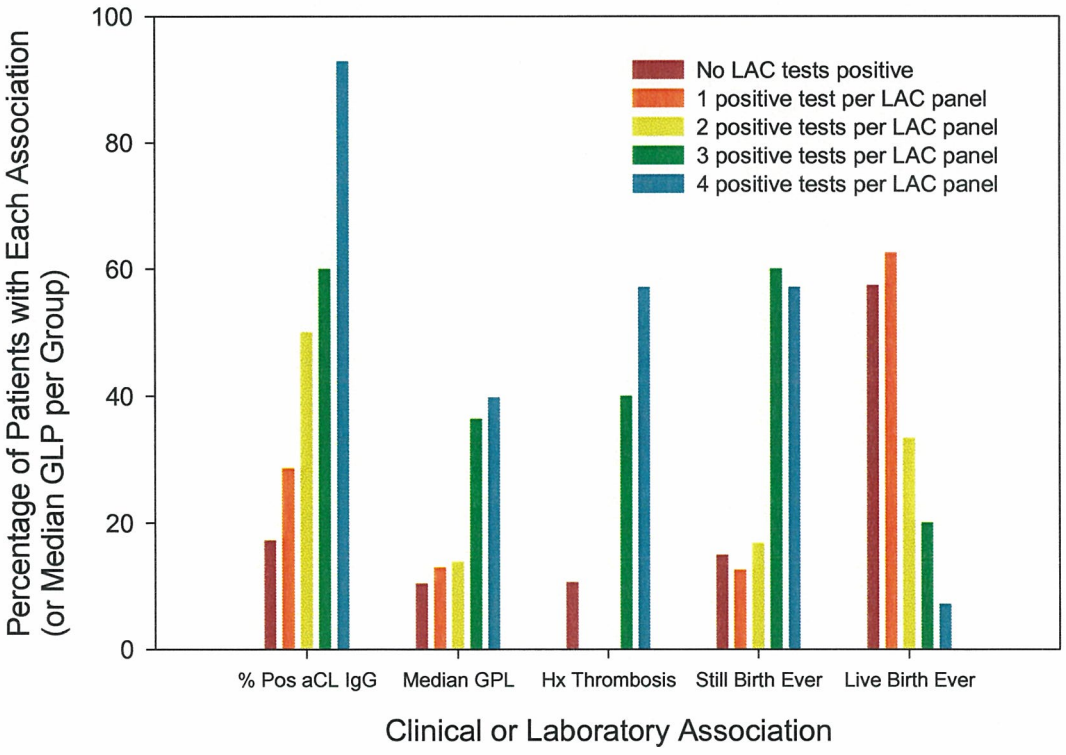
In 2004, patient accrual for a randomized, controlled clinical trial (Hep/ASA trial) was reported, and we compiled results for the 847 patients who had been screened for study participation[63]. About 15% of women had an antiphospholipid antibody (either

anticardiolipin antibody or lupus anticoagulant) and almost 60% of women had no identifiable etiology for their recurrent losses.

The association between anticardiolipin IgG and the lupus anticoagulant became evident in a study investigating the clinical events in patients with consistent and fluctuating lupus anticoagulant levels[77]. The percentage of patients positive for anticardiolipin IgG was related to the number of positive tests in the lupus anticoagulant panel. Almost all patients with all 4 tests positive for a prolonged coagulation time were also positive for anticardiolipin, and the number decreased with decreasing lupus anticoagulant positivity (Figure 1). Similarly, the median GPL for patients with all 4 lupus anticoagulant tests positive was much higher than in patients with fewer than 3 lupus anticoagulation tests positive. For 2 or fewer lupus anticoagulant tests positive, the median GPL was negative. In contrast to our findings with anticardiolipin, we found a strong correlation between number of lupus anticoagulant tests positive and a history of still birth and recurrent pregnancy loss.

The PROMISSE study is an international observational 5-year trial, funded by the National Institutes of Health (NIH) in the US, investigating the role of complement in antiphospholipid antibody-related adverse pregnancy outcome. Our laboratory is the core laboratory for lupus anticoagulant analysis. Although the trial is not yet completed, several early findings have been published, the most significant of which is that the lupus anticoagulant is the strongest predictor of serious pregnancy complications. In contrast, anti-β2GP1 antibodies and anticardiolipin antibodies, even at a cutoff of ≥ 40 GPL, are not significant predictors of pregnancy outcome[112].

Figure 1. Correlation between number of lupus anticoagulant (LAC) tests positive per panel and anticardiolipin IgG (aCL) positivity and different clinical histories[77]. The LAC was measured using a panel of 4 functional coagulation assays. Positivity in all 4 appeared to be more associated with still birth and a history of thrombosis than positivity in fewer or no LAC tests. The group of patients with 4 positive LAC tests had a higher percentage positive for aCL IgG than those with fewer positive LAC tests, and had a higher median titre than the other groups with fewer or no positive LAC tests. Conversely, patients negative for the LAC were more likely to have negative aCL, a much lower incidence of thrombosis and still birth, and a history of at least 1 live birth.



Summary of Serological Findings

• We have consistently observed that anticardiolipin antibodies are found in only a small proportion of women with recurrent pregnancy loss

Anticardiolipin IgM is a relatively non-specific finding and we were unable to use it to distinguish between women with and without recurrent pregnancy loss

• Anticardiolipin antibodies are found in other patient groups including healthy pregnant women and patients with acute parvovirus B19 infection, attesting to their presence in non-APS-related situations

• Anticardiolipin antibodies are found much more frequently in patients with a history of thrombosis than in patients with either SLE or recurrent pregnancy loss

• Anti-β2GP1 antibodies do not distinguish among patients with or without recurrent pregnancy loss or thrombosis and therefore are of limited or no value in the investigation APS

• Patients with recurrent pregnancy loss who are positive for anticardiolipin antibodies have much lower titres than those with thrombosis-related APS or SLE

• The titres in women with early recurrent pregnancy loss are so low that they do not fulfill the current classification criteria for APS and would not have done so in earlier years if quantitative antibody cut-off titres had been provided in the first set of classification criteria

• Patients with a higher number of positive lupus anticoagulant tests in a 4-test panel are more likely to have anticardiolipin antibodies and at a higher titre than those with none, 1 or 2 tests positive in the panel.

- Almost 60% of women with recurrent early pregnancy loss have no identifiable etiology

- It is the lupus anticoagulant, and not anticardiolipin antibodies, that is the strongest predictor of pregnancy complications in patients with antiphospholipid antibodies

Chapter 4. Contribution to Knowledge

Clinical Studies

Randomized Clinical Trials

Two randomized controlled clinical trials assessing treatment efficacy for recurrent pregnancy loss were conducted[55,61]. From 1988 to 1996, 1080 patients referred to our clinic for participation in the ASA/P trial. From 2000-2005, the Hep/ASA trial investigated the efficacy of low molecular weight (LMW) heparin and aspirin versus aspirin alone for treatment of recurrent pregnancy loss.

The ASA/P Trial

Over 1000 women were screened for participation in the ASA/P trial, a double-blind, placebo controlled trial comparing the efficacy of prednisone and low-dose aspirin versus placebo in women with a history of recurrent pregnancy loss. Of those, 773 women had ≥ 2 consecutive pregnancy losses, and 385 fulfilled the additional inclusion criteria: at least one positive autoantibody on at least 2 occasions and no evidence of anatomic, genetic or hormonal abnormalities. Women with 2 or more (rather than 3 or more) consecutive losses were included in our clinical trials[55, 61] as there is no difference in the distribution of known etiologies between women with 2 versus 3 losses and therefore no justification for withholding either investigation or treatment until a third loss has occurred[108-110].

After obtaining signed consent, 202 women became pregnant and were randomized into one of 4 stratified groups in the trial, based upon maternal age and gestational age of previous losses[55]. Half the women received aspirin and prednisone, the other

half received placebo. The primary outcome measure was live birth; secondary outcomes included premature birth and maternal side effects.

All women were positive for at least one autoantibody including aPL. Eighty –eight women were positive for at least one aPL: the lupus anticoagulant was present in 38 women in the treatment group and 36 in the placebo group; 6 and 14 women respectively were positive for anticardiolipin IgG. The anticardiolipin antibodies were measured using both an in-house assay[99] and 2 commercially available kits over the 8 years of patient accrual, and only women with either levels ≥ 5 standard deviations above the mean (using the in-house assay, determined by ROC analysis[37]) or above either 15 or 22 GPL (for results using the 2 different commercial kits) were considered positive. The lupus anticoagulant was found in 74 patients (37%) of the study sample which might be considered high for a population of women with recurrent pregnancy loss, but this was not an unselected sample, but instead comprised 202 women with a history of loss selected on the basis of their positivity for at least one autoantibody.

Upon completion of the trial, logistic regression analysis showed that there was no difference in response to treatment regardless of the variable tested: maternal age, history of early versus late losses, or presence of aPL. There was an increase in prematurity in the group receiving prednisone and aspirin but the birth weights were all appropriate for gestational ages, indicating no incidence of intrauterine growth restriction. As earlier studies involving aspirin during pregnancy had not resulted in any increase in prematurity, prednisone was considered to be the contributing factor in the increased rate seen in the ASA/P trial and when combined with 2 earlier

studies, these results provided sufficient data to dissuade future use of prednisone as a therapeutic option for the treatment of recurrent pregnancy loss with or without aPL.

The Hep/ASA Trial

The Hep/ASA trial was a prospective randomized, controlled study evaluating the efficacy of low molecular weight (LMW) heparin and aspirin versus aspirin alone for the treatment of recurrent pregnancy loss. LMW heparin was selected as the therapeutic intervention in this trial because it has several advantages over unfractionated heparin: increased bioavailability, more predictable dose response, less intensive coagulation monitoring, and a lower probability of causing immune-mediated thrombocytopenia in addition to decreased incidence of heparin-induced bone density loss[111]. The trial was an open label design, as the investigators did not consider daily subcutaneous placebo injections to pregnant women ethical; further, ascertainment of the primary endpoint (live birth) should not be subject to bias as a result of knowledge of treatment strategies.

A total of 859 patients were screened for participation over 5 years. Eighty-eight fulfilled classification criteria (including a history of ≥ 2 consecutive pregnancy losses and prevalence of one or more autoantibodies), signed informed consent, and were randomized into the trial once pregnancy was confirmed. Patients were stratified on the basis of presence of aPL (anticardiolipin IgG and/or IgM and/or the lupus anticoagulant) and by history of early versus late losses. Patients with one or more positive tests on a thrombophilia screen were also included. Each patient randomized into the LMW heparin group was taught to self-inject 5000 units of

Dalteparin subcutaneously once daily until 35 weeks' gestation. Oral aspirin (81 mg, enteric coated) was used in both the intervention and reference arms of the study.

The trial was designed to have a sample size of 200, but after 5 years, only 88 patients had been randomized and completed their pregnancies. A planned interim analysis built into the study design halted the study after 88 patients' pregnancies and completion of the 4-year funding period. It was revealed at that point by the steering committee that there was no difference in live birth rates between the intervention and reference groups, and that the pregnancy loss rate in the aspirin only group was much lower than anticipated[56,57]. Given the difficulty experienced in patient accrual over the study period and the lack of treatment differential, it was decided to end the trial early.

There was a 77.7% live birth rate in the group receiving LMW heparin and aspirin compared to 79.1% in the aspirin only group. There were no cases of maternal thrombosis in either treatment group during pregnancy or in the postpartum period and no significant differences in bone mineral density measurements. No difference was found when comparing outcomes of women with a history or 2 versus 3 prior pregnancy losses and there was no difference in live birth rates between women with a history of early versus late losses. There was no significant difference in the live birth rates regardless of aPL positivity. Live births were evenly distributed across IgG and IgM anticardiolipin titres, but the sample sizes were too small to confirm statistitical significance of the observation.

Results from the Hep/ASA trial supported the contention by Gates *et al*[115] that for women with recurrent pregnancy loss, aPL, and no history of thrombosis, there is insufficient evidence to support the use of thromboprophylaxis in pregnancy. Others have found a similar live birth rate in this group of patients when treated with aspirin and either LMW or unfractionated heparin[54,56-60] (Table 3). Our results confirmed an earlier trial that found no increase in live births conferred by the addition of LMW heparin to aspirin for the treatment of aPL-positive recurrent pregnancy loss[58].

Both the ASA/P and Hep/ASA trials included women with autoantibodies in addition to aPL. Anti-DNA antibodies, antinuclear antibodies, and anti-lymphocyte IgM were included in the serological criteria because recurrent pregnancy loss is a feature of systemic lupus erythematosus, and we had observed increased rates of these antibodies in our patients with recurrent pregnancy loss, particularly anti-lymphocyte IgM both with and without SLE[51,63,113]. Inclusion of autoantibodies other than aPL was felt to be important as anticardiolipin and the lupus anticoagulant are only 2 of many potential markers of recurrent pregnancy loss with an immune etiology[114,115]. It is emerging that in patients with APS, there may be an inflammatory component to the pregnancy loss that has yet to be fully elucidated[116] and may require a broadening of serological investigations to detect. We did, however, stratify our trial groups by the presence or absence of aPL, so we were able to analyze aPL subgroups without interference from the inclusion of women with other autoantibodies.

While our randomized clinical trials have been designed to evaluate treatment efficacy for women with an autoantibody or coagulation abnormality, most patients

with a history of recurrent pregnancy loss have no identifiable etiology for their losses[108]. The standard of care at our clinic for autoantibody-negative women with a history of idiopathic recurrent pregnancy loss includes low dose aspirin, close monitoring throughout pregnancy, and readily available counselling. We reviewed pregnancy outcomes at our clinic from 1995 to 2000 in women with a history of recurrent pregnancy loss who had screened negative for the full panel of autoantibodies, and who had received aspirin only during their pregnancies[115]. We identified 207 women who were negative for all autoantibodies, including antinuclear antibody, anti-DNA IgG and IgM, and anti-lymphocyte IgM as well as anticardiolipin IgG and the lupus anticoagulant. There was an 85.5% (177/207) live birth rate in the group, not statistically significantly higher than the live birth rates in women with either antiphospholipid or other autoantibodies treated with aspirin only in the Hep/ASA trial (75.0% and 82.6% respectively). This observation does not support the contention that the presence of either aPL or other lupus-related antibodies significantly affect pregnancy outcome.

Postpartum Thrombosis

Studies investigating treatment regimens for a'PL-positive pregnancy usually identify live birth rate as the primary outcome, and intrauterine growth restriction, placental infarction, prematurity and pre-eclampsia as secondary outcomes[117-120]. There is limited literature available regarding the incidence of postpartum thrombosis in this population[121]. To address this, we initiated a review of pregnancy-related thrombosis and preterm delivery in our clinic.

Over a 5-year period, patients with a history of recurrent pregnancy loss were prospectively followed throughout their pregnancies. Subsequently, a chart review was performed to collect demographic, clinical and obstetric outcome data on patients whose pregnancies had progressed to at least 27 weeks' gestation[75]. We thereby confined our analyses to those pregnancies with a significant likelihood of live birth as women with early pregnancy loss are less likely to experience pregnancy-related thrombosis[121]. There were 260 live births included in the study: 87 were positive for anticardiolipin IgG and/or the lupus anticoagulant; 173 were aPL-negative.

The only significant difference in the obstetric histories of the 2 groups was the increased incidence of prior live birth in the aPL-negative group indicating that more aPL-positive women had primary rather than secondary pregnancy loss. There was no difference in the proportion in each group with 2 versus 3 prior losses or in their ages. Prenatal and /or post partum anticoagulation therapy was given at the discretion of the attending physician. Within the aPL-positive group, 27 received aspirin only; 44 received LMW heparin and aspirin; and 16 received no treatment at all.

There was an increased rate of preterm delivery in patients with aPL compared to those without (24.0%versus 9.8%, p = 0.004, 95% confidence interval for the difference: 0.052-0.234). There were no episodes of thrombosis during any of the 260 pregnancies observed; 1 postpartum thrombotic episode occurred in the aPL-positive group. The patient had a deep vein thrombosis 3.5 weeks postpartum while receiving prophylactic LMW heparin. Interestingly, this woman did not fulfill

classification criteria for APS as she had a history of fewer than 3 early losses. In addition, it would have been difficult to attribute the specific etiology of her postpartum thrombotic episode, as it was associated with pregnancy, surgery, and immobility, 3 known risk factors for hypercoagulability in addition to the aPL-positivity.

Preterm Delivery Study

The increased rate of preterm delivery in women with aPL observed in the post-partum thrombosis study[75] confirmed earlier observations of preterm delivery in a lupus population[123]. After reviewing the pregnancies of 72 patients with SLE, we found preterm deliveries to be associated with disease and a trend toward the presence of anticardiolipin IgG but not the lupus anticoagulant. The mean weeks' gestation at delivery in women positive for anticardiolipin IgG was significantly shorter than for women negative for anticardiolipin IgG (34.9 ± 4.4 versus 37.5 ± 3.2, $p = 0.032$). In the anticardiolipin positive group, there was, however, no increase in the occurrence of intrauterine growth restriction or placental abnormalities that are characteristic of APS. We performed a literature review of lupus pregnancies and found 9 studies of lupus pregnancies from 1992 to 2002 that were observational in nature with adequate sample size and reported pregnancy outcome data. Only 2 of the 9 studies also found an association between aPL positivity and preterm delivery[124,125] without specifying anticardiolipin antibody or lupus anticoagulant.

Subsequent Pregnancies

In a study of cumulative treated pregnancies we followed 60 women from 2000 to 2004 who had more than one pregnancy after being referred to our clinic following a

history of recurrent pregnancy loss[126]. Live birth rates for women with this condition are often reported as results in a single clinical trial evaluating treatment efficacy, whereas in a "real world" setting, women frequently have more than one pregnancy subsequent to trial participation. The 60 women had a total of 184 pregnancies before attending our clinic. Fifty-one (85.0%) had a history of primary recurrent pregnancy loss; 28/60 (46.7%) were positive for aPL. In the first pregnancies treated with LMW heparin, 44 (73/3%) had a live birth. In the second treated pregnancies, 47/60 (78.3%) had a live birth. There were no differences between aPL- positive and negative women with regard to obstetric histories and subsequent pregnancy outcome. Twenty women had a third pregnancy, 6 had a fourth, and 2 had a fifth treated pregnancy. Cumulatively, the number of women with at least one live birth after 2 treated pregnancies was 54/60 (90.0%); 57/60 (95.0%) had at least one live birth after $\geq$ 2 treated pregnancies. In addition, 46/60 (76.6%) had at least 2 live births while attending our clinic.

While randomized clinical trials may provide a snapshot of therapeutic efficacy, they may not reflect the reality of treating women in a dedicated clinic setting. We found a high cumulative success rate (95.0%) over a number of pregnancies treated with LMW heparin. There was no commonality among the 5% of women who had repeated losses despite multiple treated pregnancies and we were unable to demonstrate that aPL status, prior live birth, or prior still birth were in any way associated with future pregnancy outcome.

Longterm Followup Studies

Several early followup studies reported a high incidence of thrombosis between 1 to 12 years after initial presentation with aPL- positive recurrent pregnancy loss[127,128]. Twenty years (median: 17; range: 14-21) after the first patients were enrolled in the ASA/P trial investigating the efficacy of prednisone and aspirin for recurrent pregnancy loss[55], we sent participants a self-report questionnaire to determine the incidence of subsequent thrombotic events and classification with APS[74]. When comparing the group of 56 respondents to the original trial participants, 52% had received prednisone and aspirin in the trial, 67% had a live birth in the trial, and 51% were aPL positive, none of which was significantly different from the group as a whole, indicating that the responders were representative of the original study population. Twenty-four women had 37 subsequent pregnancies resulting in 25 live births, 10 early losses, 1 stillbirth and 1 ectopic pregnancy (20/24 women had at least 1 live birth in the interval since trial participation. There have been no episodes of thrombosis even in the aPL-positive group and no one has been diagnosed with APS. Our findings are in contrast to earlier reports[127,128] but are in agreement with subsequent investigations and a recent study[129] established that asymptomatic, persistently aPL-positive patients (with no history of thrombosis or pregnancy-related events) have a low overall annual incidence of acute thrombosis and only develop vascular events when additional thrombosis risk factors are present.

Summary of Clinical Findings

- There was no difference in live birth rate between aPL- positive and negative women with recurrent pregnancy loss when treated with prednisone and aspirin or placebo.

- There was no difference in live birth rate between aPL- positive and negative women with recurrent pregnancy loss when treated with LMW heparin and aspirin or aspirin only
- There was no difference in the live birth rates between women with aPL and those with other autoantibodies when treated with aspirin
- There was no increased incidence of postpartum thrombosis in women with aPL compared to those without
- Antiphospholipid antibodies were found more frequently in preterm deliveries in women with recurrent pregnancy loss
- Anticardiolipin IgG, but not the lupus anticoagulant, was found more frequently in preterm deliveries in women with SLE although active disease at conception was a more significant predictor of adverse pregnancy outcome
- After 2 LMW heparin-treated pregnancies, 90% of women with a history of recurrent pregnancy loss had at least one live birth regardless of aPL status
- There were no thrombotic events in 55 women after a median of 17 years following initial presentation with recurrent pregnancy loss regardless of aPL status

Chapter 5. Discussion and Conclusions

Antiphospholipid syndrome (APS) is a condition that requires either (1) a documented episode of thrombosis, using objective validated evidence, of arterial, venous, or small vessel thrombosis in the absence of inflammation of the vessel wall or (2) one of a wide range of adverse obstetric outcomes, from recurrent early pregnancy loss (3 or more at < 10 weeks' gestation) or 1 late loss (≥ 10 weeks' gestation) to premature birth attributed to eclampsia, pre-eclampsia or placental insufficiency. Overlaying this diverse spectrum of clinical manifestations is the requirement for moderate to high levels of anticardiolipin or anti- β2GP1antibodies or the lupus anticoagulant. In the 25 years since APS was first described, and after many clinical and basic research studies, questions still remain regarding the significance of aPL and their association with both recurrent pregnancy loss and thrombosis[130-135].

We have reported that the presence of aPL in patients with systemic lupus erythematosus is associated with a poorer prognosis[78,130,131], even in the absence of thrombotic events and hypothesized that a vaso-occlusive factor enhances the inflammatory component in lupus, creating a condition of greater clinical concern than either entity alone.

APS has had expanding panoply of associated conditions including, but not limited to, epilepsy, transverse myelopathy, Guillain-Barré syndrome, Addisons disease, thrombocytopenia, autoimmune hemolytic anemia, idiopathic thrombocytopenic

purpura, and livedo reticularis. With each of these conditions, the presence of aPL has been reported to be associated with more severe clinical presentation[130,131], indicating that the combined presence of aPL with a comorbidity negatively affects prognosis. Indeed, aPL-positive patients with these conditions only develop vascular events when additional risk factors are present[129]. These results support the contention that aPL may be a cofactor but not a causative agent in the pathogenesis of APS-related clinical conditions[82].

Classification criteria for APS[6,7] do not differentiate between patients with severe clinical manifestations (recurrent venous and/or arterial thrombosis potentially requiring longterm if not lifelong prophylactic anticoagulation) and those with (transient) pregnancy-related events[136]. For example, compare a 30-year-old woman with a history of 3 early losses at 8 weeks' gestation and an anticardiolipin IgM of 40 MPL to a 30-year-old woman with a history of arterial thrombosis, a lupus anticoagulant, anticardiolipin IgG of >150 GPL and 1 pregnancy loss at 29 weeks' gestation accompanied by intrauterine growth restriction and placental infarction. Both patients fulfill classification for APS and may therefore be treated in the same manner in any subsequent pregnancy despite the obvious differences in the severity of their conditions. Our results over the years have indicated that the vast majority of women with a history of early pregnancy loss with aPL have a benign prognosis. We propose a cautious approach not only in terms of therapy, but also in terms of classification within APS[33,136]. This caution is supported by Branch who states that *"Though most authorities require the presence of either lupus anticoagulant or medium-to-high titer IgG anticardiolipin antibodies to make a diagnosis of antiphospholipid syndrome, in some series no more than half of the study patients*

had lupus anticoagulant and as many as 20% had only IgM anticardiolipin antibodies. It is very unlikely that patients with such disparate clinical and laboratory findings have the same autoimmune syndrome"[52]. In the past decade, there has been recognition that anticardiolipin antibodies may not actually be as strongly associated with or predictive of thrombosis or adverse pregnancy outcome as initially postulated [59,107,112,129,136-139].

Numerous potential aPL-mediated disease mechanisms, both at the molecular level and demonstrated in murine models, have been proposed (Table 2, p.13). However, evidence supporting the pathogenicity of aPL in humans remains elusive and understanding the cause of tissue injury, particularly fetal loss in APS, remains incomplete. While the placental insufficiency and resultant intrauterine growth restriction seen in a subset of preterm deliveries and still births may be related to a pathological condition beyond the normal hypercoagulability of pregnancy, anticardiolipin antibodies may also be nothing more than a complicating epiphenomenon[28].

Intuitively, given the classification criteria for APS, one would assume at least a consistent correlation between the presence of aPL and clinical manifestations of the syndrome. However, this is not the case. The majority of women with recurrent early pregnancy loss are negative for aPL as are the majority of patients presenting with deep vein thrombosis and stroke[64]. Conversely, a minority of women with uneventful pregnancies has been shown to be positive for aPL and in a followup of asymptomatic, persistently aPL-positive patients with no clinical manifestations of APS, investigators found a low overall annual incidence of thrombosis and no benefit

accrued by prophylactic treatment with low dose aspirin[65]. It is of interest to note that treatment does not differ for patients with recurrent thromboembolism regardless of their aPL status. It is the recurrent thrombotic event itself, and not the presence of aPL, that determines the treatment regimen[66]. It is apparent, therefore, that aPL are neither necessary nor sufficient for the development of clinical manifestations of APS.

The lack of both a compelling pathogenic mechanism and the absence of a consistent association between aPL and clinical manifestations may explain the inconclusive outcomes from clinical trials evaluating varied treatment regimes for aPL-positive women with a history of recurrent pregnancy loss. We and others have observed that when aPL are present with early recurrent pregnancy loss, the live birth rate is similar to that with aPL-negative women with RPL, regardless of therapeutic intervention and regardless of titres of anticardiolipin antibodies or how the lupus anticoagulant is measured (Table 3). In addition, we have noted no increase in pregnancy-related vascular thrombosis and there is a growing body of evidence that there is no increase in longterm sequelae following recurrent pregnancy loss.

Nevertheless, women with early recurrent pregnancy loss and aPL fulfill current criteria although they may never have any non-pregnancy-related events. Classification with APS, which is categorized as an uninsurable condition by the insurance industry[86-88], precludes obtaining longterm care insurance and complicates application for life insurance. Patients are thus burdened with lifelong health and

financial implications regardless of the fact that most of the women with a history of early pregnancy loss will never have any non-obstetric manifestations of the disease.

There are 3 distinct disease entities of APS: primary (PAPS: in the absence of a comorbidity); secondary (SAPS: with a pre-existing comorbidity); and catastrophic (CAPS: simultaneous multi-organ failure with small vessel occlusion). "Seronegative APS", first proposed in 1997[137], describes patients with a typical idiopathic clinical picture suggestive of APS, but without evidence of aPL in their serum. The concept of seronegative disease is not new. Clinically active, serologically quiescent lupus has been described for more than 30 years[140,141]. However, unlike classification with APS, classification with SLE comprises fulfillment of at least 4 criteria among a number of clinical and laboratory elements, not a specific requirement for a specific autoantibody[142]. This latter variant of seronegative APS is both puzzling and intriguing: classification with this syndrome has a definite requirement for a positive aPL on at least 2 occasions. In the absence of aPL, isn't the idiopathic clinical event just the clinical event? Perhaps by introducing this seronegative variant, the investigators have inadvertently undermined the very nature of the putative syndrome by declaring that a heretofore-essential classification element is not necessarily required.

Extensive studies focusing on recurrent pregnancy loss in patients have enabled the following conclusions:

1. The majority of women with early recurrent pregnancy loss are negative for aPL.
2. The majority of patients seen in our clinic with early recurrent pregnancy loss who

are positive for aPL never experience any of the non-pregnancy-related clinical conditions included in the classification criteria for APS.

3. aPL, especially anticardiolipin antibodies, are neither necessary nor sufficient for the development of early recurrent pregnancy loss.

These conclusions indicate the need for redefinition of APS. Removal of early recurrent pregnancy loss from the classification criteria would address the increasing body of evidence that this clinical manifestation is distinct from late loss or early delivery with placental infarction[143]. Alternatively, the introduction of a new subset, possibly termed "transient APS", would distinguish anticardiolipin-positive patients with limited, pregnancy-related morbidity and no longterm sequelae from those with recurrent episodes of venous and/or arterial thrombosis requiring lifelong prophylactic anticoagulation. Just as drug-induced lupus is a completely distinct entity from SLE, and recognized as a temporary condition with a resolution of symptoms upon drug withdrawal[144], similarly, "transient APS" might be recognized as designation that would more appropriately reflect the limited nature and more positive longterm prognosis for women with early recurrent pregnancy loss and anticardiolipin antibodies.

APS is a syndrome built upon a foundation with two pillars of classification criteria (laboratory and clinical). It has already been shown that even the absence of a pillar (seronegative APS) has not deterred investigators from classifying patients with this syndrome, resulting, perhaps, in inappropriate longterm health and insurance consequences. There is sufficient evidence to question the validity of the current criteria for APS, particularly with regard to the inclusion of early recurrent pregnancy

loss and anticardiolipin positivity. As Branch so aptly put it in 1998[52], *"a stated or implicit diagnosis of antiphospholipid syndrome in such a wide variety of women is scientifically unsound and clinically dangerous"*. It is perhaps time to review the classification criteria for APS: every year this situation goes uncorrected means hundreds if not thousands of women are bearing an unnecessary diagnostic burden.

References

1. Lahita RG. The role of sex hormones in systemic lupus erythematosus. Curr Opin Rheumatol 1999;11:352-6.

2. Clark CA, Spitzer KA, Laskin CA. Decrease in pregnancy loss rates in patients with systemic lupus erythematosus over a 40-year period. J Rheumatol 2005;32:1709-12.

3. Johansson EA, Lassus A. The occurrence of circulating anticoagulants in patients with syphilitic and biologically false positive antilipoidal antibodies. Blood 1955;10:691

4. Boey ML, Colaco CB, Gharavi AE, Slekon KB, Loizou S, Hughes GR. Thrombosis in systemic lupus erythematosus: striking association with the presence of circulating lupus anticoagulant. Br Med J (Clin Res Ed). 1983;287:1021-3.

5. Harris EN, Gharavi AE, Boey ML, et al. Anticardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. Lancet. 1983;26;1211-4.

6. Wilson WA, Gharavi AE, Koike T, et al. International consensus statement of preliminary classification criteria for definite antiphospholipid syndrome: report of an international workshop. Arthritis Rheum 1999;42:1309-11.

7. Miyakis S, Lockshin MD, Atsumi T, et al. International consensus statement on an update of the classification criteria for definite antiphospholipid syndrome (APS). J Thromb Haemost. 2006;4:295–306

8. Gharavi AE, Pierangeli SS. Origin of antiphospholipid antibodies: induction of aPL by viral peptides. Lupus 1998; 7 Suppl 2: S52-4

9. Uthman IW, Gharavi AE. Viral infections and antiphospholipid antibodies. Semin Arthritis Rheum 2002;31:256-63.

10. Gharavi AE, Pierangeli SS, Harris EN. Viral origin of antiphospholipid antibodies: endothelial cell activation and thrombus enhancement by CMV peptide induced APL antibodies. Immunobiology 2003;207:37-42.

11. Harel M, Aron-Maor A, Sherer Y, Blank M, Shoenfeld Y. The infectious etiology of the antiphospholipid syndrome: links between infection and autoimmunity. Immunobiology 2005;210:743-7.

12. Ahserson RA, Cervera R. Antiphospholipid antibodies and infections. Ann Rheum Dis 2003;62:388-93.

13. Merrill JT. Do antiphospholipid antibodies develop for a purpose? Curr Rheumatol Rep 2006;8:109-13.

14. Sherer Y, Blank M, Shoenfeld Y. Antiphospholipid syndrome (APS): where does it come from? Best Tract Res Clin Rheumatol 2007;21:1071-8.

15. Khamashta MA, Asherson RA, Hughes GR. Possible mechanisms of action of the antiphospholipid binding antibodies. Clin Exp Rheumatol 1989;7 Suppl 3:S85-9.

16. Triplett DA. Antiphospholipid antibodies: proposed mechanisms of action. Am J Reprod Immunol 1992;28:211-5.

17. Field SL, Brighton TA, McNeil HP, Cherterman CN. Recent insights into antiphospholipid antibody-mediated thrombosis. Baillieres Best Pract Res Clin Haematol 1999;12:407-22.

18. Pierangeli SS, Harris EN. In vivo models of thrombosis for the antiphospholipid syndrome. Lupus 1996;5:247-51.

19. Rand JH. Antiphospholipid antibody syndrome: New insights on thrombogenic mechanisms. Am J Med Sci 1998;316:142-51.

20. Kandiah DA, Sali A, Sheng Y, et al. Current insights into the "antiphospholipid" syndrome: clinical, immunological, and molecular aspects. Adv Immunol 1998;70:507-63.

21. Ieko M, Sawada KI, Koike T, et al. The putative mechanism of thrombosis in antiphospholipid syndrome: impairment of the protein C and the fibrinolytic systems by monoclonal anticardiolipin antibodies. Semin Thromb Hemost 1999;25:503-7.

22. Angles-Cano E, Guillin MC. Antiphospholipid antibodies and the coagulation cascade. Rheum Dis Clin North Am 2001;27:573-86.

23. Singh AK. Immunopathogenesis of the antiphospholipid antibody syndrome: an update. Curr Opin Nephrol Hypertens 2001;10:355-8.

24. Radway-Bright EL, Ananc M, Isenberg DA. Animal models of the antiphospholipid syndrome. Rheumatology (Oxford) 1999;38:591-601.

25. Salmon JE, Girardi G. The role of complement in the antiphospholipid syndrome. Curr Dir Autoimmun 2004;7:113-48.

26. Weiler H. Tracing the molecular pathogenesis of antiphospholipid syndrome. J Clin Invest 2008;118:3276-78.

27. Girardi G, Redecha P, Salmon JE. Heparin prevents antiphospholipid antibody-induced fetal loss by inhibiting complement activation. Nat Med 2004;10:1222-1226.

28. Rand JH. Molecular pathogenesis of the antiphospholipid syndrome. Circ Res 2002;90:29-37.

29. Sherer Y, Shoenfeld Y. Antiphospholipid antibodies: are they pro-atherogenic or an epiphenomenon of atherosclerosis? Immunobiology 2003;207:13-6.

30. Hughes GRV. Hughes syndrome (the antiphospholipid syndrome): Ten clinical lessons. Autoimmun Rev 2008;7:262-6.

31. Meroni RL. Pathogenesis of the antiphospholipid syndrome: An additional example of the mosaic of autoimmunity. J Autoimmun 2008;30:99-103.

32. Derksen RH, Kater L. Lupus anticoagulant: revival of an old phenomenon. Clin Exp Rheumatol 1985;3:349-57.

33. Keil LB, Galazka M, el-Kadi HS, Erickson EH Jr, DeBari VA. Binding of beta 2 glycoprotein I to activated polystyrene and its recognition by human IgG autoantibodies. Biotechnol Appl Biochem 1995;22:305-13.

34. Galli M, Comfurius P, Maassen C, et al. Anticardiolipin antibodies (ACA) directed not to cardiolipin but to a plasma protein cofactor. Lancet 1990;335:1544-7.

35. Laskin CA, Soloninka CA. Anti-cardiolipin antibodies: Smoking gun or smoke screen? [editorial] J Rheumatol 1988;15:7-9.

36. Petri M. Diagnosis of antiphospholipid antibodies. Rheum Dis Clin North Am. 1994;20:443-69

37. Soloninka CA, Laskin CA, Wither J, Wong D, Bombardier C, Raboud J. Clinical utility and specificity of anti-cardiolipin antibodies. J Rheumatol 1991;18:1849-55

38. Bertolaccini ML, Atsumi T, Escudero Contreras A, Khamashta MA, Hughes GR. The value of IgA antiphospholipid testing for diagnosis of antiphospholipid (Hughes) syndrome in systemic lupus erythematosus. J Rheumatol. 2001;28:2637-43.

39. Guglielmone H, Vitozzi S, Elbarcha O, Fernandez E. Cofactor dependence and isotype distribution of anticardiolipin antibodies in viral infections. Ann Rheum Dis. 2001 May;60(5):500-4.

40. Soloninka CA, Laskin CA. Anti-cardiolipin antibodies in acute human parvovirus B19 infection. Arthritis Rheum 1995;38 Suppl:S400.

41. Reber G, Boehlen F, de Moerloose P. Technical aspects in laboratory testing for antiphospholipid antibodies: is standardization an impossible dream? Semin Thromb Hemost 2008;34:340-6.

42. Pierangeli SS, Harris EN. A quarter century in anticardiolipin antibody testing and attempted standardization has led us to here, which is? Semin Thromb Hemost 2008;34:313-28.

43. Bruce IN, Clark-Soloninka CA, Sptizter KA, Gladman D, Rowitz MB, Laskin CA. Prevalence of antibodies to b2-glycoprotein I in systemic lupus erythematosus and their association with antiphospholipid syndrome criteria: a single center study and literature review. J Rheumatol 2000;27:2833-7.

44. Galli M, Reber G, De Moerloose P, De Groot PG. Invitation to a debate on the serological criteria that define the antiphospholipid syndrome. J Thromb Haemost 2008; 6: 399–401.

45. Favaloro EJ, Wong RC. Laboratory testing and identification of antiphospholipid antibodies and the antiphospholipid syndrome: a potpourri of problems: a compilation of possible solutions. Semin Thromb Hemost 2008;34:389-410.

46. Wong RC, Favaloro EJ. A consensus approach to the formulation of guidelines for laboratory testing and reporting of antiphospholipid antibody assays. Semin Thromb Hemost 2008;34;361-72.

47. Triplett DA, Harris EN. Antiphospholipid antibodies and reproduction. Am J Reprod Immunol 1989;21:123-31.

48. Love PE, Santoro SA. Antiphospholipid antibodies: anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders. Prevalence and clinical significance. Ann Intern Med 1990; 112:682-98.

49. Gleicher N. Autoantibodies in normal and abnormal pregnancy. Am J Reprod Immunol 1992;28:269-73.

50. Soloninka CA, Laskin CA, Bombardier C, Wong D, Spitzer K, Fielding LJ.. Anti-cardiolipin IgG in women with recurrent fetal loss. Arthritis Rheum 1995;38 Suppl:S390.

51. Laskin CA, Spitzer KA, Soloninka CA, Bombardier C. Circulating autoantibodies in women with unexplained recurrent pregnancy losses: results from an evaluation of 783 women. Arthritis Rheum 1997;40 Suppl:S304.

52. Branch DW. Antiphospholipid antibodies and reproductive outcome: the current state of affairs. J Reprod Immunol 1998;38:75-87.

53. Harger JH, Laifer SA, Bontempo FA, Senich LA, Church C. Low-dose aspirin and prednisone treatment of pregnancy loss caused by lupus anticoagulants. J Perinatol 1995;15:463-9.

54. Cowchock FS, Reese EA, Balaban D,, Branch DW, Plouffe L. Repeated fetal losses associated with antiphospholipid antibodies: a collaborative randomized trial comparing prednisone with low dose heparin treatment. 1992 May;166(5):1318-23.

55. Laskin CA, Bombardier C, Hannah ME, Mandel FP, Ritchie JWK, Farewell V, Farine D, Sptizer KA, Fielding LJ, Soloninka CA, Yeung M. Prednisone and aspirin in women with autoantibodies and unexplained recurrent fetal loss. N Engl J Med 1997;337:148-53.

56. Rai R, Cohen H, Dave M, Regan L. Randomised controlled trial of aspirin and aspirin plus heparin in pregnant women with phospholipid antibodies (or antiphospholipid antibodies). BMJ 1997;314:253-7.

57. Kutteh WH. Antiphospholipid antibody-associated recurrent pregnancy loss: treatment with heparin and low-dose aspirin is superior to low-dose aspirin alone. Am J Obstet Gynecol 1996;174:1584-9.

58. Farquharson RG, Quenby S, Greaves M. Antiphospholipid syndrome in pregnancy: a randomized controlled trial of treatment. Obstet Gynecol 2002;100:408-13.

59. Franklin RD, Kutteh WH. Antiphospholipid antibodies (APA) and recurrent pregnancy loss: treating a unique APA positive population. Human Reproduction 2002;17:2981-5.

60. Noble LS, Kutteh WH, Lashey N, Franklin RD, Herrada J. Antiphospholipid antibodies associated with recurrent pregnancy loss: prospective, multicenter, controlled pilot study comparing treatment with low-molecular-weight heparin versus unfractionated heparin. Fertil Steril 2005;83:684-90.

61. Laskin CA, Spitzer KA, Clark CA, et al. Low molecular weight heparin and aspirin for recurrent pregnancy loss: Results from the randomized, controlled Hep/ASA trial. J Rheumatol 2009;36:279-87.

62. Empson MB, Lassere M, Craig JC, Scott JR. Prevention of recurrent miscarriage for women with antiphospholipid antibody or lupus anticoagulant. Cochrane Database of Systematic Reviews 2005; CD002859.

63. Clark CA, Laskin CA, Ginsberg JS, et al. Screening results for 847 women attending a recurrent pregnancy loss clinic. Arthritis Rheum 2004;50 Suppl:S71.

64. Edwards CJ, Hughes GR. Hughes syndrome (the antiphospholipid syndrome): 25 years old. Mod Rheumatol 2008;18:199-24.

65. Opatrny L, David M, Kahn SR, Shrier I, Rey E. Association between antiphospholipid antibodies and recurrent fetal loss in women without autoimmune disease: a metaanalysis. J Rheumatol 2006;33:2214-21.

66. Lim W, Crowther MA, Eikelboom JW. Management of antiphospholipid antibody syndrome: a systematic review. JAMA 2006;295:1050-7.

67. Cervera R, Khamashta MA, Shoenfeld Y, et al. Morbidity and mortality in the antiphospholipid syndrome during a 5-year period: a multicenter prospective study of 1000 patients. Ann Rheum Dis 2008; 68:1428-32.

68. Shoenfeld Y, Meroni PL, Toubi E. Antiphospholipid syndrome and systemic lupus erythematosus: are they separate entities or just clinical presentation on the same scale? Curr Opin Rheumatol 2009;21:495-500.

69. Erkan D, Harrison MJ, Levy R, et al. Aspirin for primary thrombosis prevention in the antiphospholipid syndrome: r randomized, double-blind, placebo-controlled trial in asymptomatic antiphospholipid antibody-positive individuals. Arthritis Rheum 2007;56:2382-91.

70. Silver RM, Draper ML, Scott JR, Lyon JL, Reading J, Branch DW. Clinical consequences of antiphospholipid antibodies: an historic cohort study. Obstet Gynecol 1994;83:372-7.

71. Ercan D, Merrill JT, Yazici Y, Sammaritano L, Buyon JP, Lockshin MD. High thrombosis rate after fetal loss in antiphospholipid syndrome: effective prophylaxis with aspirin. Arthritis Rheum 2001; 44:1466-69.

72. Tincani A, Taglietti M, Biasini C, Frassi M, Gorla R, Balestrieri G. Thromboembolic events after fetal loss in patients with antiphospholipid syndrome: comment on the article by Erkan et al. Arthritis Rheum 2002 46:1126-7.

73. Quenby S, Farqharson RG, Dawood F, Hughes AM, Topping J. Recurrent miscarriage and long-term thrombosis risk: a case-control study. Human Reprod 2005;20:1729-32.

74. Clark CA, Spitzer KA, Laskin CA. Longterm followup of ASA/P trial participants. Arthritis Rheum 2009; in press.

75. Clark CA, Spitzer KA, Crowther MA, Nadler,JN, Laskin MD, Waks JA, Laskin CA. Incidence of post-partum thrombosis and preterm delivery in women with anti-phospholipid antibodies and recurrent pregnancy loss. J Rheumatol 2007;34:992-6.

76. James AH. Venous thromboembolism in pregnancy. Arteriosclerosis, Thrombosis, and Vascular Biology. 2009;29:326-31.

77. Clark CA, Spitzer KA, Laskin CA. Comparison of clinical events in patients with consistent and fluctuating lupus anticoagulant results. Arthritis Rheum 2005; 52 Suppl: S601.

78. Laskin CA, Clark CA, Spitzer KA. Antiphospholipid syndrome in SLE: Is the whole greater than the sum of the parts? Rheum Dis Clin North Am. 2005;31:255-72.

79. Danowski A, de Azevedo MN, de Souza Papi JA, Petri M. Determinants of risk for venous and arterial thrombosis in primary antiphospholipid syndrome and in antiphospholipid syndrome with systemic lupus erythematosus. J Rheumatol. 2009;36:1195-9.

80. James AH, Abel DE, Brancazio LR. Anticoagulants in pregnancy. Ob Gyn Survey 2005;61:59-69.

81. Tuthill JI, Khamashta MA. Management of antiphospholipid syndrome. J Autoimmun 2009;33:92-8.

82. Tanne D, O'Olhaberriague L, Schultz LR, Salowich-Palm L, Sawaya KL, Levine SR. Anticardiolipin antibodies and their associations with cerebrovascular risk factors. Neurology 1999;52:1368-73.

83. Miesbach W, Scharrer I, Asherson RA. High titres of IgM-antiphospholipid antibodies are unrelated to pathogenicity in patients with non-Hodgkin's lymphoma. Clin Rheumatol 2007;26:95-7.

84. Lynch A, Silver R, Emlen W. Antiphospholipid antibodies in healthy pregnant women. Rheum Dis Clin North Am 1997;23:55-70.

85. Proven A, Bartlett RP, Moder KG, Clinical importance of positive test results for lupus anticoagulant and anticardiolipin antibodies. Mayo Clin Proc 2004;79:467-75.

86. Assurant Health. Medical and non-medical underwriting guidelines. Accessed on 21 October 2009. Available at: http://www.grpbenltd.com/webres/File/docs_for_agent_portal/Underwriting%20Field%20Guide%20(cond%20list).pdf

87. Genworth Financial. Longterm care insurance underwriting guide. Accessed on 21 October 2009. Available at: http://sdiconline.com/PDF/Genworth_LTC_Field_Underwriting_Manual507.pdf

88. Mutual of Omaha Insurance Company. Longterm care insurance underwriting guide. Accessed on 21 October 2009. Available at: http://www.nyltcb.com/brokers/pdfs/Mutual_Omaha_Underwriting_Guide.pdf

89. Branch WE, Scott JR, Kochenour NK, et al. Obstetric complications associated with the lupus anticoagulant. . N Engl J Med 1985:313:1322-26.

90. Hughes GRV. Thrombosis, abortion, cerebral disease and lupus anticoagulant. BMJ 1983;287:1088-9.

91. Eswaran K, Rosen SE. Recurrent abortions, thromboses, and a circulating anticoagulant. Am J Obstet Gynecol 1985;151:751-52.

92. Petri M, Golbus M, Anderson R, et al. Antinuclear antibody, lupus anticoagulant, and anticardiolipin antibody in women with idiopathic habitual abortion. Arthritis Rheum 1987;30:601-6.

93. Lockshin MD, Qamar T, Druzin ML, Goei S. Antibody to cardiolipin, lupus anticoagulant, and fetal death. J Rheumatol. 1987;4:259-62.

94. Lockshin MD, Druzin ML, Goei S, Qamar T, Magid MS, Jovanovic L, Ferenc M Antibody to cardiolipin as a predictor of fetal distress or death in pregnant patients with systemic lupus erythematosus. N Engl J Med. 1985;313:152-6.

95. Harris EN, Chan JK, Asherson RA, Aber VR, Gharavi AE, Hughes GR. Thrombosis, recurrent fetal loss, and thrombocytopenia. Predictive value of the anticardiolipin antibody test. Arch Intern Med. 1986;146:2153-6.

96. Meyer O, Cyna L, Borda-Iriarte O, et al. [Antiphospholipid antibodies, thrombosis and lupus disease. Value of the assay of anticardiolipin antibodies by the ELISA technique.] Rev Rhum Mal Osteoartic. 1985;52:297-305.(French).

97. Ichikawa Y, Kobayashi N, Kawada T, Anticardiolipin antibodies by an enzyme-linked immunosorbent assay: fundamental studies on the conditions for antigen-application and specificity of the assay. Tokai J Exp Clin Med. 1989;14:103-12.

98. Isenberg DA, Colaco CB, Dudeney C, Todd-Pokropek A, Snaith ML. The relationship of anti-DNA antibody idiotypes and anticardiolipin antibodies to disease activity in systemic lupus erythematosus. Medicine 1986;65:45-55.

99. Lafer EM, Rauch J, Andrzejewski JR, et al. Polyspecific monoclonal lupus antibodies reactive with both polynucleotides and phospholipids. J Exp Med 1981;153:897-909.

100. Clark CA, Spitzer KA, Laskin CA. Prevalence and utility of anti-cardiolipin IgA and anti-phospholipid antibodies in screening for recurrent pregnancy loss. Canadian Fertility and Andrology National Scientific Meeting, Jasper AL, CAN. November 2004.

101. Escalente A. Brey RL, Mitchell BD, Dreiner U. Accuracy of anticardiolipin antibodies in identifying a history of thrombosis among patients with systemic lupus erythematosus. Am J Med 1995;98:559-65.

102. Bertolaccini ML, Atsumi T, Escudero Contreras A, Khamashta MA, Hughes GR. The value of IgA antiphospholipid testing for diagnosis of antiphospholipid (Hughes) syndrome in systemic lupus erythematosus. J Rheumatol 2001;28:2637-43.

103. Soloninka CA, Anderson MJ, Laskin CA. Anti-DNA and antilymphocyte antibodies during acute infection with human parvovirus B19. J Rheumatol 1989;16:777-81.

104. Stimmler MM, Quismorio FP Jr, McGehee WG, Boylen T, Sharma OP. Anticardiolipin antibodies in acquired immunodeficiency syndrome. Arch Intern Med. 1989 149:1833-5.

105. Clark-Soloninka CA, Bruce IN, Spitzer KA, Nadler JN, Laskin CA. Anti-B2 glycoprotein I and anti-cardiolipin antibodies distinguish clinical subsets within the anti-phospholipid antibody syndrome. Arthritis Rheum 1998;41 Suppl : S168.

106. Clark CA, Spitzer KA, Nadler JN, Laskin CA. Low aCL IgG titres in recurrent pregnancy loss signal the need for redefining APS. Arthritis Rheum 2002; 46 Suppl:S50.

107. Salmon JE, Kim M, Guerra M, Clark C, Laskin CA, Branch W, Sammaritano L, Petri M, Merrill JT, Lockshin M, Buyon JP. Absence of lupus anticoagulant is a strong predictor of uncomplicated pregnancy in patients with APL antibodies. Arthritis Rheum 2008;58 Suppl : S403.

108. Brigham SA, Conlon C, Farquharson RG. A longitudinal study of pregnancy outcome following idiopathic recurrent miscarriage. Human Reprod 1999;14:2868-71.

109. American College of Obstetricians and Gynecologists. Management of recurrent early pregnancy loss. ACOG Practice Bulletin No. 24, 2001;24:1-8.

110. Jaslow CR, Carney JL, Kutteh WH. Diagnostic features identified in 1020 women with 2 versus 3 or more recurrent pregnancy losses. Fertil Steril 2009; Mar 30. [Epub ahead of print]

111. Hawkins D, Evans J. Minimizing the risk of heparin –induced osteoporosis during pregnancy. Expert Opin Drug Saf 205;4:583-90.

112. Gates S, Brockenlhurst P, Davis LJ. Prophylaxis for venous thromboembolic disease in pregnancy and the early postpartum period. Cochrane Database Syst Rev 2002;2:CD001689.

113. Laskin CA, Spitzer KA, Soloninka CA, Bombardier C. Circulating autoantibodies in women with unexplained recurrent pregnancy losses: results from an evaluation of 783 women. Arthritis Rheum 1997;40 Suppl:S304.

114. Clark CA, Spitzer CA, Laskin CA. The Hep/ASA trial. J Rheumatol 2010;37: 203.

115. Laskin CA, Clark CA, Spitzer KA. Efficacy of low dose ASA administration in autoantibody-negative women with a history of recurrent pregnancy loss. Fertil Steril, 2005;84 Suppl: S447.

116. Hughes G. Hughes syndrome: the antiphospholipid syndrome – a clinical overview. Clin Rev Allergy Immunol 2007;32:3-12.

117. Geis W, Branch DW. Obstetric implications of antiphospholipid antibodies; pregnancy loss and other complications. Clin Obstet Gynecol 2001;44:2-10.

118. Derksen RHWM, de Groot PG, Nieuwenhuis HK, Christianes GCML. How to treat women with antiphospholipid antibodies in pregnancy? Ann Rheum Dis 2001;60:1-3.

119. Tricolo G, Ferrante A, Ciccia F, et al. Randomized study of subcutaneous low molecular weight heparin plus aspirin versus intravenous immunoglobulin in the treatment of recurrent fetal loss associated with antiphospholipid antibodies. Arthritis Rheum 2003;48:728-31.

120. Derksen RHWM, Khamashta MA, Branch DW. Management of the obstetric antiphospholipid syndrome. Arthritis Rheum 2004;50:1028-39.

121. Salonen Ros H, Lichtenstein P, Bellocco R, Petersson G, Cnattinguius S. Increased risks of circulatory diseases in late pregnancy and pueriperium. Epidemiology 2001;12:456-60.

122. James AH. Prevention and management of venous thromboembolism in pregnancy. Am J Med 2007;120 suppl: S26-34.

123. Clark CA, Spitzer KA, Nadler JN, Laskin CA. Preterm deliveries in women with systemic lupus erythematosus. J Rheumatol 2003;30:2127-32.

124. Cortes-Hernandez J, Ordi-Ros J, Paredes F, Casellas M, Castillo R, Milardell-Tarres M. Clinical predictors of fetal and maternal outcome in systemic lupus erythematosus: a prospective study of 103 pregnancies. Rheumatology (Oxford) 2002;41:643-50.

125. Le Huong D, Wechsler B, Vauthier-Brouzes D, et al. Outcome of planned pregnancies in systemic lupus erythematosus: a prospective study of 62 pregnancies. Br J Rheumatol 1997;36:772-7.

126. Clark CA, Spitzer KA, Laskin CA. Successive pregnancy outcomes with low molecular weight heparin and ASA. 7th International Congress on SLE and Related Conditions, New York, NY. 9-13 May 2004; M42B.

127. Silver RM, Draper ML, Scott JR, Lyon JL, Reading J, Branch DW. Clinical consequences of antiphospholipid antibodies: an historic cohort study. Obstet Gynecol 1994;83:372-7.

128. Ercan D, Merrill JT, Yazici Y, Sammaritano L, Buyon JP, Lockshin MD. High thrombosis rate after fetal loss in antiphospholipid syndrome: effective prophylaxis with aspirin. Arthritis Rheum 2001; 44:1466-69.

129. Erkan D, Harrison MJ, Levy R, et al. Aspirin for primary thrombosis prevention in the antiphospholipid syndrome: a randomized, double-blind, placebo-controlled trial in asymptomatic antiphospholipid antibody positive individuals. Arthritis Rheum 2007;56:2382-91.

130. Laskin CA, Clark CA, Spitzer KA. Pregnancy and autoimmune rheumatic disease. In: Legato MJ, editor. Principles of Gender Specific Medicine 2nd Edition. Elsevier Press: San Diego: 2009: 627-44.

131. Laskin CA, Spitzer KA, Clark CA. Pregnancy and reproductive concerns in systemic lupus erythematosus. In: Lahita RG, editor. Systemic Lupus Erythematosus, 5th Edition. Wiley Medical: New York; 2010:(in press).

132. Spitzer KA, Murphy K, Crowther M, Clark CA, Laskin CA. Post-partum management of women at increased risk of thrombosis – results of a Canadian pilot survey. J Rheumatol 2006;33:2222-6.

133. Ruiz-Irastorza G, Khamashta MA. Stroke and antiphospholipid syndrome: the treatment debate. Rheumatology (Oxford) 2005;44:971-4.

134. Van Goor MP, Alblas CL, Leebeek RW, Koudstaal PJ, Dippel DW. Do antiphospholipid antibodies increase the longterm risk of thrombotic complications in young patients with a recent TIA or ischemic stroke? Acta Neurol Scand 2004;109:410-5.

135. Levine SR, Brey RL, Tilley BC, et al. Antiphospholipid antibodies and subsequent thrombo-occlusive events in patients with ischemic stroke. JAMA 2004;291:576-84.

136. Clark CA, Spitzer KA, Laskin CA. The spectrum of the antiphospholipid syndrome: a matter of perspective. J Rheumatol 2001;28:1939-41.

137. Miret C, Cervera R, Reverter JC, et al. Antiphospholipid syndrome without antiphospholipid antibodies at the time of the thrombotic event: transient "seronegative" antiphospholipid syndrome? Clin Exp Rheumatol 1997;15:541-4.

138. Bertolaccini ML, Khamashta MA. Laboratory diagnosis and management challenges in the antiphospholipid syndrome. Lupus. 2006;15:172-8.

139. Baker WE Jr, Bick RL. The clinical spectrum of antiphospholipid syndrome. Hematol Oncol Clin North Am 2008;22:33-52.

140. Gladman DD, Urowitz MB, Keystone EC. Serologically active clinically quiescent systemic lupus erythematosus: a discordance between clinical and serological features. Am J Med 1979;66:210-5.

141. Gladman DD, Hirani N, Ibanez D, Urowitz MD. Clinically active serologically quiescent systemic lupus erythematosus. J Rheumatol 2003;30:1960-2.

142. Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271-7.

143. Branhma K, Hunt BJ, GermainS, et al Pregnancy outcome in different clinical phenotypes of antiphospholipid syndrome. Lupus 2009; Nov 6. [Epub ahead of print]

144. Vedove CD, Del Giglio M, Schena D, Girolomoni G. Drug-induced lupus erythematosus. Arch Dermatol Rec 2009;301:99-105.

Glossary of Acronyms

aCL	Anti-cardiolipin antibodies
aPL	Anti-phospholipid antibodies
APS	Antiphospholipid syndrome
ASA/P	Aspirin and prednisone clinical trial
B2GP1	Beta-2 glycoprotein 1
ELISA	Enzyme-linked immunosorbent assay
GPL	IgG anticardiolipin units
Hep/ASA	Low molecular weight heparin and aspirin clinical trial
IUGR	Intrauterine growth restriction
LAC	Lupus anticoagulant encompassing: DilPT: dilute prothrombin time DRVVT: dilute Russell's viper venom time PTT-LA: LAC-sensitive partial thromboplastin time KCT: kaolin clotting time
LMW	Low molecular weight (heparin)
MPL	IgM anticardiolipin units
ROC	Receiver operator characteristics
RPL	Recurrent pregnancy loss
SLE	Systemic lupus erythematosus
UF	Unfractionated (heparin)

Appendix 1

Candidate's Submitted References

Note surname variations:
Soloninka: 1988-1997
Clark-Soloninka:1998-2000
Clark: 2001-present

1. Laskin CA, Soloninka CA. Anti-cardiolipin antibodies: Smoking gun or smoke screen? [editorial] J Rheumatol 1988;15:7-9.

2. Laskin CA, Soloninka CA, Chin W, Droppo L. Association of a non-cytotoxic anti-lymphocyte antibody with unexplained recurrent fetal loss in patients with sub-clinical autoimmunity. Arthritis Rheum 1989;32: 1 Suppl:R5.

3. Soloninka CA, Laskin CA, Wither J, Wong D, Bombardier C, Raboud J. Clinical utility and specificity of anti-cardiolipin antibodies. J Rheumatol 1991;18:1849-55

4. Soloninka CA, Laskin CA. Anti-cardiolipin antibodies in acute human parvovirus B19 infection. Arthritis Rheum 1995;38 Suppl:S400: presentation to the American College of Rheumatology National Scientific Meeting, San Francisco, CA; 26 October 1995.

5. Soloninka CA, Laskin CA, Bombardier C, Wong D, Spitzer K, Fielding LJ.. Anti-cardiolipin IgG in women with recurrent fetal loss. Arthritis Rheum 1995;38 Suppl:S390.

6. Laskin CA, Spitzer KA, Soloninka CA, Bombardier C. Circulating autoantibodies in women with unexplained recurrent pregnancy losses: results from an evaluation of 783 women. Arthritis Rheum 1997;40 Suppl:S304.

7. Laskin CA, Bombardier C, Hannah ME, Mandel FP, Ritchie JWK, Farewell V, Farine D, Sptizer KA, Fielding LJ, Soloninka CA, Yeung M. Prednisone and aspirin in women with autoantibodies and unexplained recurrent fetal loss. N Engl J Med 1997;337:148-53.

8. Clark-Soloninka CA, Bruce IN, Spitzer KA, Nadler JN, Laskin CA. Anti-B2 glycoprotein I and anti-cardiolipin antibodies distinguish clinical subsets within the anti-phospholipid antibody syndrome. Arthritis Rheum 1998;41 Suppl : S168.

9. Bruce IN, Clark-Soloninka CA, Spitzer KA, Gladman D, Morowitz MB, Laskin CA. Prevalence of antibodies to b2-Glycoprotein I in systemic lupus erythematosus and their association with antiphospholipid syndrome criteria: a single center study and literature review. J Rheumatol 2000;27:2833-7.

10. Clark CA, Spitzer KA, Laskin CA. The spectrum of the antiphospholipid syndrome: a matter of perspective. J Rheumatol 2001;28:1939-41.

11. Clark CA, Spitzer KA, Nadler JN, Laskin CA. Low aCL IgG titres in recurrent pregnancy loss signal the need for redefining APS. Arthritis Rheum 2002; 46 Suppl:S50.

12. Clark CA, Spitzer KA, Nadler JN, Laskin CA. Preterm deliveries in women with systemic lupus erythematosus. J Rheumatol 2003;30:2127-32.

13. Clark CA, Laskin CA, Ginsberg JS, et al. Screening results for 847 women attending a recurrent pregnancy loss clinic. Arthritis Rheum 2004;50 Suppl:S71.

14. Clark CA, Spitzer KA, Laskin CA. Successive pregnancy outcomes with low molecular weight heparin and ASA. 7th International Congress on SLE and Related Conditions. New York, NY. 9-13 May 2004; M42B.

15. Clark CA, Spitzer KA, Laskin CA. Comparison of clinical events in patients with consistent and fluctuating lupus anticoagulant results. Arthritis Rheum 2005; 52 Suppl: S601.

16. Laskin CA, , Spitzer KA. Antiphospholipid syndrome in SLE: Is the whole greater than the sum of the parts? Rheum Dis Clin North Am. 2005;31:255-72.

17. Clark CA, Sptizer KA, Laskin CA. Decrease in pregnancy loss rates in patients with systemic lupus erythematosus over a 40-year period. J Rheumatol 2005;32:1709-12.

18. Clark CA, Spitzer KA, Crowther MA, Nadler,JN, Laskin MD, Waks JA, Laskin CA. Incidence of post-partum thrombosis and preterm delivery in women with anti-phospholipid antibodies and recurrent pregnancy loss. J Rheumatol 2007;34:992-6.

19. Laskin CA, Spitzer KA, Clark CA, Crowther MR, Ginsberg JS, Hawker GA, Kingdom JC, Barrett J, Gent M. Low molecular weight heparin and aspirin for recurrent pregnancy loss: results from the randomized, controlled Hep/ASA trial. J Rheumatol 2009;36:279-87.

20. Clark CA, Spitzer KA, Laskin CA. Longterm followup of ASA/P trial participants. Arthritis Rheum 2009;Suppl.

21. Laskin CA, Clark CA, Spitzer KA. Pregnancy and autoimmune rheumatic disease. In: Legato MJ, editor. Principles of Gender Specific Medicine 2nd Edition. Elsevier Press: San Diego: 2009: 627-44.

22. Clark CA, Spitzer CA, Laskin CA. The Hep/ASA trial [letter]. J Rheumatol 2010;37: 203.

23. Laskin CA, Spitzer KA, Clark CA. Pregnancy and reproductive concerns in systemic lupus erythematosus. In: Lahita RG, editor. Systemic Lupus Erythematosus, 5th Edition. Wiley Medical: New York; 2010: (in press).

Appendix 2.

Statements of Co-Authorship

Statement of Co-Authorship

I, Ian N. Bruce, confirm that Christine A. Clark made the following contributions to our study, *Prevalence of antibodies to β2-glycoprotein I in systemic lupus erythematosus and their association with antiphospholipid antibody syndrome criteria: a single centre study and literature review (published in the* Journal of Rheumatology 2000;27:2833-7).

Chris' contributions:

- Assisted in developing study design
- Generated study results and interpreted and analysed study data in partnership with co-investigators
- Performed literature review
- Co-authored manuscript for publication with first author
- Solicited feedback and comments on drafts from co-investigators

I give my permission for this study to be used as a part of Chris' application for the PhD by Published Works programme.

Respectfully yours,

Ian Bruce

7th July 2008

Ian N Bruce, MD FRCP
Reader and Honorary Consultant in Rheumatology,
arc Epidemiology Unit
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The University of Manchester,
Manchester, UK

Statement of Co-Authorship

I, Carl A. Laskin, confirm that Christine A. Clark made the following contributions to the following peer reviewed and published studies. Please be aware that I am the senior author on all of these publications, and that Chris has been running my research laboratory since June 1983 (excluding the period from 1984-5 when she was in graduate school at UCL, UK).

---

1. Laskin CA, **Soloninka CA**, Chin W, Droppo L. *Association of a non-cytotoxic anti-lymphocyte antibody with unexplained recurrent fetal loss in patients with sub-clinical autoimmunity.* Arthritis Rheum 1989;32: 1 Suppl:R5.

Chris' contributions:

- Contributed to study design
- Generated study results and interpreted and analysed study data in partnership with senior author
- Solicited comments on drafts of abstract from co-authors
- Prepared figures and poster presentation in collaboration with co-authors

2. **Soloninka CA,** Laskin CA, Wither J, Wong D, Bombardier C, Raboud J. *Clinical utility and specificity of anti-cardiolipin antibodies.* J Rheumatol 1991;18:1849-55

Chris' contributions:

- Contributed to study design
- Generated study results and interpreted and analysed study data in partnership with co-investigators
- Prepared figures and co-authored manuscript with senior author
- Solicited feedback and comments on drafts of the manuscript from co-investigators.
- Responded to reviewers' comments prior to publication

3. **Soloninka CA**, Laskin CA. *Anti-cardiolipin antibodies in acute human parvovirus B19 infection.* Arthritis Rheum 1995;38 Suppl:S400.

Chris' contributions:

- Conceptualized study design
- Generated study results
- Interpreted and analyzed study data with senior author
- Co-authored abstract with senior author
- Prepared and gave presentation at ACR meeting

4. **Soloninka CA**, Laskin CA, Bombardier C, Wong D, Spitzer K, Fielding LJ. *Anti-cardiolipin IgG in women with recurrent fetal loss.* Arthritis Rheum 1995;38 Suppl:S390.

Chris' contributions:

- Contributed to study design
- Generated study results and interpreted and analysed study data in partnership with senior author
- Solicited comments on drafts of abstract from co-authors
- Prepared figures and poster presentation in collaboration with co-authors

5. Laskin CA, Spitzer KA, **Soloninka CA**, Bombardier C. *Circulating autoantibodies in women with unexplained recurrent pregnancy losses: results from an evaluation of 783 women.* Arthritis Rheum 1997;40 Suppl:S304.

Chris' contributions:

- Contributed to study design
- Generated and analyzed study results
- Collaborated with co-authors on interpretation of results
- Solicited comments on drafts of abstract from co-authors
- Prepared figures and poster presentation, solicited reviews from co-authors

6. Laskin CA, Bombardier C, Hannah ME, Mandel FP, Ritchie JWK, Farewell V, Farine D, Sptizer KA, Fielding LJ, **Soloninka CA**, Yeung M. *Prednisone and aspirin in women with autoantibodies and unexplained recurrent fetal loss.* N Engl J Med 1997;337:148-53.

Chris' contributions:

- Contributed to methodology section of study design
- Generated laboratory results
- Collaborated with co-authors on interpretation of results
- Contributed to preparation of manuscript for submission
- Prepared response to reviewers with senior author and co-author (Spitzer)
- Prepared final manuscript with senior author and co-author

7. **Clark-Soloninka CA**, Bruce IN, Spitzer KA, Nadler JN, Laskin CA. *Anti-B2 glycoprotein I and anti-cardiolipin antibodies distinguish clinical subsets within the anti-phospholipid antibody syndrome.* Arthritis Rheum 1998;41 Suppl : S168.

Chris' contributions:

- Contributed to study design
- Generated study results, interpreted and analysed data with co-investigators
- Performed literature review
- Co-authored abstract
- Prepared presentation for international meeting

8. **Clark CA**, Spitzer KA, Nadler JN, Laskin CA. *Low aCL IgG titres in recurrent pregnancy loss signal the need for redefining APS.* Arthritis Rheum 2002; 46 Suppl:S50.

Chris' contributions:

- Conceptualized study design with senior author
- Generated study results
- Collated retrospective data; cleaned and finalized data set

- Performed data analysis
- Interpreted analysed data with coauthors
- Authored abstract for publication and prepared presentation for international meeting

9. **Clark CA**, Spitzer KA, Nadler JN, Laskin CA. *Preterm deliveries in women with systemic lupus erythematosus.* J Rheumatol 2003;30:2127-32.

Chris' contributions:
- Conceptualized and designed study in collaboration with senior author
- Collated retrospective data; cleaned and finalized data set.
- Performed data analysis
- Interpreted analysed data in partnership with co-investigators
- Co-authored study report and responded to reviewers' comments

10. **Clark CA**, Laskin CA, Ginsberg JS, et al. *Screening results for 847 women attending a recurrent pregnancy loss clinic.* Arthritis Rheum 2004;50 Suppl:S71.

Chris' contributions:
- Collaborated on study design
- Generated laboratory screening results
- Interpreted analysed data in partnership with co-investigators
- Co-authored abstract for publication and prepared presentation for international meeting

11. **Clark CA**, Spitzer KA, Laskin CA. *Successive pregnancy outcomes with low molecular weight heparin and ASA.* 7th International Congress on SLE and Related Conditions, New York, NY. 9-13 May 2004; M42B.

Chris' contributions:
- Conceptualized and designed study in collaboration with senior author
- Collated retrospective data; cleaned and finalized data set.
- Performed data analysis
- Prepared abstract and presentation for international meeting

12. **Clark CA**, Spitzer KA, Laskin CA. *Comparison of clinical events in patients with consistent and fluctuating lupus anticoagulant results.* Arthritis Rheum 2005; 52 Suppl: S601.

Chris' contributions:
- Conceptualized and designed study
- Collated retrospective data; cleaned and finalized data set.
- Performed data analysis
- Interpreted analysed data in partnership with co-investigators
- Authored report for publication and presentation at international conference

13. **Clark CA**, Sptizer KA, Laskin CA. *Decrease in pregnancy loss rates in patients with systemic lupus erythematosus over a 40-year period.* J Rheumatol 2005;32:1709-12.

Chris' contributions:

- Conceptualized and designed study
- Collated retrospective data; cleaned and finalized data set
- Performed literature review
- Performed statistical analysis
- Interpreted data in partnership with co-authors
- Authored manuscript
- Responded to reviewers comments prior to publication

14. **Clark CA,** Spitzer KA, Crowther MA, Nadler,JN, Laskin MD, Waks JA, Laskin CA. *Incidence of post-partum thrombosis and preterm delivery in women with anti-phospholipid antibodies and recurrent pregnancy loss.* J Rheumatol 2007;34:992-6.

Chris' contributions:

- Conceptualized and designed study in partnership with co-investigators
- Collated retrospective data; cleaned and finalized data set.
- Performed data analysis
- Interpreted analysed data with co-investigators
- Authored study report for publication
- Solicited feedback and comments on drafts from co-investigators
- Responded to reviewers' comments prior to publication

15. Laskin CA, Spitzer KA, **Clark CA**, Crowther MR, Ginsberg JS, Hawker GA, Kingdom JC, Barrett J, Gent M. *Low molecular weight heparin and aspirin for recurrent pregnancy loss: results from the randomized, controlled Hep/ASA trial.* J Rheumatol 2009;36:279-87.

Chris' contributions:

- Collaborated on study design
- Generated autoimmune laboratory data
- Cleaned and finalized data set
- Interpreted and analyzed data with coauthors
- Coauthored study report and collated co-investigators' comments
- Coauthored response to reviewers' comments prior to publication

16. **Clark CA**, Spitzer KA, Laskin CA. *Longterm followup of ASA/P trial participants.* Arthritis Rheum 2009; Suppl.

Chris' contributions:

- Collaborated on study design
- Generated, cleaned and finalized data set
- Analyzed data
- Interpreted data with co-authors
- Prepared abstract and presentation for international meeting

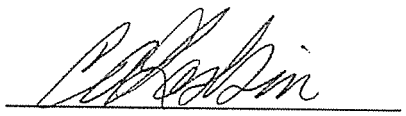
17. Laskin CA, **Clark CA**, Spitzer KA. *Pregnancy and autoimmune rheumatic disease.* In: Legato MJ, editor. Principles of Gender Specific Medicine 2nd Edition. Elsevier Press: San Diego: 2009: 627-44.

Chris' contributions:

- Performed literature review
- Prepared references
- Co-authored manuscript with senior author
- Edited final draft of the manuscript

In addition to these among other peer-reviewed and published studies which she has co-authored over the years, Chris was also a co-author of 2 invited editorials: *Anti-cardiolipin antibodies: Smoking gun or smokescreen?* (J Rheumatol 1988;15:7-9.) and *The spectrum of the antiphospholipid syndrome: A matter of perspective* (J Rheumatol, 2001; 28:1939-41). She collaborated with me on the concept development and the authorship, and had the final edit of both manuscripts.

I give my permission for these publications to form part of her submission for the PhD by Published Works programme.



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Obstetrics and Gynecology, and Immunology,
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Date

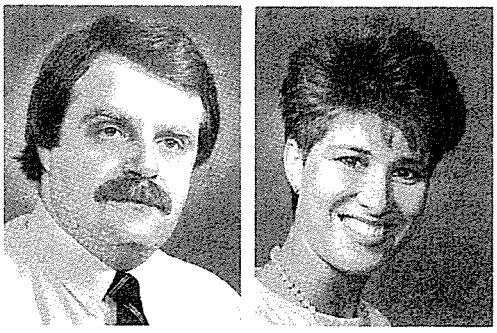
Appendix 3.

Full texts of published works submitted in support of the Context Statement.

Documents 1-23

Editorial

Anticardiolipin Antibodies: Smoking Gun or Smoke Screen?



Over the past 5 years the presence of anticardiolipin antibodies (aCL) in patients with systemic lupus erythematosus (SLE) and women with recurrent fetal loss has generated a great deal of research activity[1,2]. One merely has to examine the pages of this journal over the past year to verify this claim. In this issue, articles by Weidmann, *et al*[3] Hazeltine, *et al*[4] and Koskela, *et al*[5] highlight the excitement that has been generated by the study of these antibodies and the controversies that have arisen regarding the significance of their presence. It is appropriate to step back and evaluate the importance of detection of such antibodies and examine the fervent assertions regarding their association with clinical disorders.

Anticardiolipin antibodies (aCL) are not a "new" discovery. Their description dates back at least to 1941 by Pangborn[6]. The first evidence of their significance can be attributed to Johansson and Lassus[7]. They noted the association of antibodies to phospholipids with the circulating anticoagulant described earlier in lupus patients with falsely positive tests for syphilis[8,9]. These associations with the lupus anticoagulant and the biologically falsely positive VDRL (BFP) have brought aCL to the fore. Indeed, both of the papers in this issue of the *Journal* substantiate at least the association between aCL and the BFP. However, it is important to note that although almost all patients with BFP are positive for aCL, the reverse is far from true. The majority of patients found to be positive for aCL do not have the BFP[10-12]. Is there something significant about one of these groups of patients?

Traditionally, the importance of the BFP has been its association with the lupus anticoagulant. Whereas the BFP has been prominent in the diagnosis of SLE[13], the lupus anticoagulant is significant in identifying those lupus patients at high risk for coagulopathies manifested by recurrent venous and arterial thrombosis[14-16] and more recently, recurrent fetal loss[17-19]. Fifteen percent of patients with SLE possess the lupus anticoagulant. However, almost 50% of lupus pregnancies are characterized by fetal loss[20]. Clearly, the high prevalence of fetal wastage in patients with SLE must be due to factors other than the lupus anticoagulant. Recently, the discrepancy between the prevalence of the lupus anticoagulant and the higher prevalence of fetal wastage appeared to have been resolved when an association between the presence of aCL and recurrent fetal loss was found[11,21]. These authors purported that the presence of aCL is actually a better predictor of fetal wastage than the lupus anticoagulant. This last observation has now become the "claim to fame" of aCL and been the subject of several symposia and workshops. It is also this observation that has sparked the most controversy.

There are few who would disagree that the presence of lupus anticoagulant has a high association with fetal loss[11,17-20,22,23]. Unfortunately, many may prematurely adhere to the view that there is an equally strong association between aCL and recurrent fetal loss. The prevalence of aCL in patients with SLE varies between 11 and 61%[24,25]. Although most studies have shown a significant association between recurrent fetal loss and the presence of aCL, a note of caution must be exercised in relying too heavily on the predictive value of this antibody.

If aCL is predictive of fetal loss, pregnant patients with this antibody may benefit from treatment. However, based upon published data, this antibody is actually not predictive of fetal loss. In the few studies that include the often omitted control group of pregnant normal women, aCL are found in moderately high frequency when compared to nonpregnant, healthy women[11]. Our own preliminary results find aCL in 22% of a sample of 34 pregnant healthy women. Petri, *et al*[25] found a frequency of 11% in both patients with habitual abortion and nonpregnant controls, but the levels were higher in the aborters. We would therefore contend that aCL are a relatively common occurrence in normal pregnancies but may be more frequent and in higher titer in those with recurrent fetal loss. However, who could predict which patients are at risk of fetal loss until after the fact? It is obvious that controlled prospective studies must be done before we dare treat those patients who have this antibody. As part of these controlled studies, perhaps a lesson might be learned from Weidmann, *et al* who suggest in this issue of the *Journal* that the isotype of aCL and other antiphospholipid antibodies may be of crucial importance.

A major factor in accounting for the controversies regarding aCL prevalence and associations is the variety of assay systems used in the different laboratories. The aCL assay is usually performed by radioimmunoassay or ELISA[11,24,26]. Regardless of the specific method, the assay is noted for the

difficulty in dissolving the cardiolipin antigen as well as the problem of binding the soluble antigen to the assay plates. Once these feats are performed, you have to deal with the problem of assay background levels. Although Weidmann, *et al* seem to have escaped the problem of assay background, Hazeltine, *et al* were not so fortunate. The upper limit in the normal population obtained by the latter investigators is inordinately high for the assay but is similar to that obtained by others[24-26]. Furthermore, Lockshin, *et al* state "the serum factor identified by the enzyme-linked immunosorbent assay [is presumptive] 'antibody to cardiolipin' and should not be interpreted to mean 'specific antibody to cardiolipin'." It is therefore not surprizing that the collective prevalence data lack consistency and that controversy concerning the predictive value of this antibody has arisen. It is clear that standards could be derived by the pooling of antisera among investigators similar to that strategy followed in the HLA workshops.

In addition to thrombosis and fetal loss, other clinical manifestations have been associated with the presence of aCL. Hazeltine, *et al* confirm previous observations of the association of aCL with lupus anticoagulant activity, the BFP, thrombocytopenia[14,24,27], and possibly an association with Coombs' positive hemolytic anemia. Weidmann, *et al* note a negative association of aCL with renal disease. In contrast, Hazeltine, *et al* found no correlation with disease activity. Various investigators have found an inconsistent association with disease activity which may be attributable to the idiosyncrasies of the various assay systems.

Conclusions regarding the significance of aCL are best determined by gathering a consensus among investigators and clinicians. First, there is some association between aCL and recurrent fetal loss, the importance of which remains to be determined. Secondly, aCL are frequently found in patients with recurrent fetal loss, but the predictive value of these antibodies is too questionable a criterion upon which to base any therapeutic decisions. Thirdly, aCL do not appear to be related to disease activity, but may be associated with certain clinical manifestations. Lastly, but perhaps of greatest importance, assay systems must be standardized and specificities assured before the controversy surrounding aCL can be resolved. The time for descriptive studies involving this antibody and possible clinical associations has passed. We now require results from well controlled, prospective studies and randomized trials to determine the true significance of aCL.

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REFERENCES

1. Harris EN, Loizou S, Englert H, *et al:* Anticardiolipin antibodies and lupus anticoagulant. *Lancet 1984;*2:1099.
2. Harris EN, Gharavi AE, Hughes GRV: Anti-phospholipid antibodies. *Clin Rheum Dis 1985;*11:591-609.
3. Weidman CE, Wallace DJ, Peter JB, *et al:* Significance of false positive syphilis reactions and antiphospholipid antibody isotopes in systemic lupus erythematosus. *J Rheumatol 1988;*15:74-79.
4. Hazeltine M, Rauch J, Danoff D: Antiphospholipid antibodies in systemic lupus erythematosus: evidence of an association with positive Coombs' and hypocomplementemia. *J Rheumatol 1988;*15:80-86.
5. Koskela P, Vaarals O, Makitalo R, *et al:* Significance of false positive syphilis reactions and anticardiolipin antibodies in a nationwide series of pregnant women. Cardiolipin antibodies and pregnancy. *J Rheumatol 1988;*15:70-73.
6. Pangborn MC: A new serologically active phospholipid from beef heart. *Proc Soc Exp Biol Med 1941;*48:484-486.
7. Johansson EA, Lassus A: The occurrence of circulating anticoagulants in patients with syphilitic and biologically false positive antilipoidal antibodies. *Blood 1955;*10:691.
8. Ley AB, Render GG, Sorenson CW, *et al:* Idiopathic hypoprothrombinemia associated with hemorrhagic diathesis and the effect of vitamin K. *Blood 1951;*6:740.
9. Conley CL, Hartmann RC: Hemorrhagic disorder caused by circulating anticoagulant in patients with disseminated lupus erythematosus. *J Clin Invest 1952;*31:621-622.
10. Colaco CB, Male DK: Anti-phospholipid antibodies in syphilis and a thrombotic subset of SLE: distinct profiles of epitope specificity. *Clin Exp Immunol 1985;*59:449-456.
11. Lockshin MD, Druzin ML, Goei S, *et al:* Antibody to cardiolipin as a predictor of fetal distress or death in pregnant patients with systemic lupus erythematosus. *N Engl J Med 1985;*313:152-156.
12. Harris EN, Hughes GRV, Gharavi AE: Antiphospholipid antibodies: An elderly statesman dons new garments. *J Rheumatol 1987;*(suppl 13)14:208-213.
13. Tan EM, Cohen AS, Rothfield NF, *et al:* The 1982 revised criteria for classification of SLE. *Arthritis Rheum 1982;*25:1271-1277.
14. Boey ML, Colaco CB, Gharavi AE, *et al:* Thrombosis in systemic lupus erythematosus: striking association with the presence of circulating lupus anticoagulant. *Br Med J 1983;*287:1021-1023.
15. Gastineau DA, Kazmier FJ, Nichols WL, *et al:* Lupus anticoagulant: An analysis of the clinical and laboratory features of 219 cases. *Am J Hematol 1985;*19:265-275.
16. Mueh JR, Herbst KD, Rappaport SI: Thrombosis in patients with the lupus anticoagulant. *Ann Intern Med 1980;*92:156-159.
17. Hughes GRV: Thrombosis, abortion, cerebral disease and lupus anticoagulant. *Br Med J 1983;*287:1088-1089.
18. Eswaran K, Rosen SW: Recurrent abortions, throboses, and a circulating anticoagulant. *Am J Obstet Gynecol 1985;*151:751-752.
19. Branch WD, Scott JR, Kochenour NK, *et al:* Obstetric complications associated with the lupus anticoagulant. *N Engl J Med 1985;*313:1322-1326.
20. Zurier R: Systemic lupus erythematosus and pregnancy. In: Lahita RG, ed. *Systemic Lupus Erythematosus*, New York: John Wiley & Sons. 1987:541-554.
21. Derue G, Englert H, Harris EN, *et al:* Fetal loss in systemic lupus: association with anticardiolipin antibodies. *J Obstet Gynecol 1985;*2:207-209.

22. Nilsson IM, Astedt B, Hedner U, *et al:* Intrauterine death and circulating anticoagulant ("antithromboplastin"). *Acta Med Scand 1975;*197:153–159.
23. Firkin BG, Howard MA, Radford N: Possible relationship between lupus inhibitor and recurrent abortion in young women. *Lancet 1980;*2:366.
24. Harris EN, Gharavi AE, Boey ML, *et al:* Anticardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in SLE. *Lancet 1983;*2:1211–1214.
25. Petri M, Golbus M, Anderson R, *et al:* Antinuclear antibody, lupus anticoagulant, and anticardiolipin antibody in women with idiopathic habitual abortion. *Arthritis Rheum 1987;*30:601–606.
26. Loizou S, McCrea JD, Rudge AC, *et al:* Measurement of anti-cardiolipin antibodies by an enzyme-linked immunosorbent assay (ELISA): standardization and quantitation of results. *Clin Exp Immunol 1985;*62:728–745.
27. Derksen RHWM, Kater L: Lupus anticoagulant: Revival of an old phenomenon. *Clin Exp Rheumatol 1985;*3:349–357.

1N

ASSOCIATION OF A NON-CYTOTOXIC ANTI-LYMPHOCYTE ANTIBODY WITH UNEXPLAINED RECURRENT FETAL LOSS IN PATIENTS WITH SUBCLINICAL AUTOIMMUNITY. Carl A. Laskin, Christine A. Soloninka, Warren Chin, Lisa Droppo. Toronto General Hospital, University of Toronto, Toronto, Ont., Canada.

We have previously reported that in a group of 22 patients with Unexplained recurrent fetal loss (RFL), 20 patients had at least 1 autoantibody. An IgM anti-lymphocyte antibody was found to be the most sensitive and specific marker associated with Unexplained RFL demonstrated in 18/22 patients in the latter category but rarely detected in the Explained RFL and pregnant normal controls. We have now characterized the anti-lymphocyte antibody and further analyzed its antigenic specificity.

In 8/13 patients with Unexplained RFL found to have serum anti-lymphocyte antibody binding using an ELISA, none demonstrated cytotoxicity in a microcytotoxicity assay. In contrast, 23/23 SLE patients demonstrated the anti-lymphocyte antibody by ELISA, with 19 of these found to be cytotoxic. Specificity studies revealed that the anti-lymphocyte antibody found in patients with Unexplained RFL is highly cross-reactive with cell surface component(s) found on human RBC, platelets, and trophoblastic tissue. In addition, the antibody also detected a component on the surface of murine spleen cells and thymocytes. No cross-reactivity was demonstrated with either native or denatured DNA or cardiolipin antigens.

A Western blot analysis of the serum anti-lymphocyte antibody performed on a human lymphocyte cell lysate subjected to SDS-PAGE, revealed 5 bands where binding occurred at: 35 KDa, 25 KDa, 17 KDa, 14 KDa, and 13 KDa.

Based upon these studies, we conclude: 1) anti-lymphocyte antibodies found in patients with Unexplained RFL are non-cytotoxic in contrast to those found in patients with SLE; 2) these non-cytotoxic antibodies are not specific for lymphocytes but rather bind to a cell surface component common to many human and murine cells; 3) the cell surface components to which this anti-lymphocyte antibody binds is similar to that described for the antibody to lupus associated membrane protein.

2N

EVIDENCE FOR THE PRESENCE OF A BRAIN-SPECIFIC ANTIGEN RECOGNIZED BY ANTIBODIES IN THE SERUM OF PATIENTS WITH CNS-SLE. Mark D. Horowitz, Daniel Rosenbluth, D. Steve Kohtz, Saul Puszkin, and Harry Spiera, Mount Sinai School of Medicine, New York, N.Y.

An antibody was identified in the serum of a patient with overt CNS-SLE which specifically bound to a brain synaptic plasma membrane antigen. This antigen (Lp50) appears to be a 50 KD integral membrane glycoprotein, detected in brain and was not detected in tissue extracts of cardiac muscle, striated muscle, liver, kidney, or pancreas. We obtained sera from ten patients with CNS-SLE. CNS manifestations included seizures, psychosis, transient or permanent focal neurologic deficits, and severe, unremitting headaches. Our control groups included 15 patients with SLE without CNS disease, 8 patients with other forms of connective tissue disease (PSS, PM/DM, RA, rheumatoid vasculitis (RV), and Still's disease) and 3 normal control volunteers. The sera were screened by Western Blot technique for binding activity to Lp50 patially purified from bovine brain synaptic plasma membrane. Sera from 21 patients showed moderate or strong reactivity to Lp50. Nine had CNS lupus, 8 had SLE without CNS manifestations, and one each had RA, RV, PSS, and PM. In total 90% (9/10) of patients with CNS lupus and 53% (8/15) of patients with SLE without CNS manifestations demanstrated reactivity to Lp50. None of the three normal controls did so.

The antigen described is distinct from ribosomal p-protein as dearibed by Bonfa et al. The function of Lp50 antigen and the significance of anti-Lp50 antibodies are unknown and await further study.

3N

THROMBOSPONDIN SYNTHESIS AND SECRETION BY ACTIVATED POLYMORPHONUCLEAR LEUKOCYTES IN SYNOVIAL FLUID. André D. Beaulieu, Monique La Fleur, Christophe Kreis, Inflammation and Immunology-Rheumatology Research Unit, Université Laval, Quebec City, Canada.

Few studies have addressed the question of protein synthesis and secretion by polymorphonuclear leukocytes (PMN) at sites of inflammation. Using ^{35}S-methionine metabolic labeling, we studied de novo synthesis and secretion of proteins by activated PMN. Two different sources of activated PMN were studied. We used PMN isolated from inflammatory synovial fluid of patients with rheumatoid and psoriatic arthritis and comparisons were made with non-activated PMN isolated from the peripheral blood of the same patient. We also analyzed protein synthesis and secretion by glycogen activated PMN obtained from the peritoneal cavity of rabbits. Again comparisons were made with non-activated PMN isolated from the peripheral blood of the same rabbit. Cells were labeled for a period of 16 hours and supernatants were analyzed by 1-dimensional gel electrophoresis. In both models, the activated PMN showed a marked increase in the synthesis and secretion of thrombospondin as identified by a mouse monoclonal antibody. This increased production by activated cells paralleled a similar increase in production of another extracellular matrix and cell adhesion protein, fibronectin. We conclude that activated PMN at sites of inflammation such as chronic synovitis are able to produce in increased amounts at least two tissue matrix proteins, thrombospondin and fibronectin. The role that these proteins play in inflammation and PMN physiology remains to be determined.

4N

HYDROXYAPATITE PSEUDOPODAGRA: A SYNDROME OF YOUNG WOMEN. *Adel Fam, Joel Rubenstein. Sunnybrook Medical Ctr(SMC), U of Toronto, ONT,CanadaM4N3M5*

Acute calcific periarthritis(ACP) is a disorder characterized by acute periarticular inflammation associated with juxtaarticular hydroxyapatite calcific deposits. ACP of 1st metatarsophalangeal (MTP) joint or "hydroxyapatite pseudopodagra" is rare. In the past year, we have treated 3 women ages 26,28,&31 yrs with ACP of 1st MTP jts. This prompted us to review 3 prior cases seen at SMC.

Unexpectedly, all 6 pts were women, mean age 35(26-53yrs). One pt had onset of acute attack during pregnancy. Presentation was characterized by acute onset of pain, swelling & erythema of the great toe indistinguishable from that of gouty podagra. All cases were initially mistaken for acute gout but serum urate & biochemical studies were normal. Attempted 1st MTP arthrocentesis in 3/6 pts yielded a dry tap in 2(no crystals in needle-tip)&2 drops of sterile,crystal-negative, non-inflammatory synovial fluid(800WBC/mm3)in one. Acute episodes responded to NSAIDs; mean duration of attack 10d.

Literature review uncovered 10 additional pts with ACP of 1st MTP jts; 8 of them were females,mean age 30(range12-49yrs). The F:M ratio for the entire group of 16 pts was 7:1,compared to a ratio of approx. 1:1 for ACP in general. Radiographic findings revealed amorphous, periarticular calcific deposits of varying shape,size & density around the 1st MTP jts. Calcifications were located medial(7/12),lateral(3/12) & plantar(2/12) to the joint(location not specified in remaining 4 pts). Followup films(11/16) showed disappearance of calcification over 2-16wk

In conclusion:1)Acute calcific periarthritis is an uncommon cause of pseudopodagra. 2)Hydroxyapatite pseudopodagra is a disorder prodominantly of premenopausal women & should be considered in the differential diagnosis of acute 1st MTP monoarthritis. 3)Radiographs of the 1st MTP joint in such pts require careful examination for periarticular calcification.

5N

PROTECTIVE EFFECTS OF CORTICOSTEROIDS ON CARTILAGE LESIONS AND OSTEOPHYTE FORMATION IN THE EXPERIMENTAL DOG MODEL OF OSTEOARTHRITIS. Jean-Pierre Pelletier, Johanne Martel-Pelletier, Research Center Notre-Dame Hospital, University of Montreal, Montreal, Canada.

The *in vivo* effects of corticosteroids on the progression of osteoarthritic lesions (cartilage erosions and osteophyte formation) were examined in 12 dogs in whom the anterior cruciate ligament had been sectioned. Six of these dogs received oral prednisone (0.25 mg/kg/day) and 6 received intra-articular injections of corticosteroids (triamcinolone hexacetonide, 5 mg) every four weeks. Twelve other operated dogs received no treatment. All dogs were sacrificed 8 weeks after surgery. Of the 15 normal controls, 4 received intra-articular injections of triamcinolone hexacetonide. Operated untreated dogs developed significant cartilage lesions on their femoral condyles and tibial plateaus and presented prominent osteophytes. Operated dogs treated with either oral or intra-articular corticosteroids demonstrated a significant reduction in osteophyte size ($p < 0.006$; $p < 0.04$). Whereas cartilage erosions on femoral condyles were observed in 25% of the non-treated dogs, erosions were found in only 8% of the dogs receiving oral prednisone; and in none of the dogs treated intra-articularly. In addition, for both oral and intra-articular administrations, a significant reduction in tibial plateau lesion size was found, compared to operated untreated dogs ($p < 0.04$; $p < 0.0002$). Histological findings from the operated animals revealed that corticosteroids significantly reduce the severity of osteoarthritic structural changes of the cartilage on both medial and lateral femoral condyles and tibial plateaus, with the exception of the lateral plateaus of animals treated with oral corticosteroids. Electron microscopy studies demonstrated no evidence of increased cell degeneration or death with steroids. Intra-articular corticosteroids had no deleterious effects on normal articular cartilage. These results indicate that glucocorticoids administered either orally or intra-articularly are effective therapeutic drugs against the development of osteoarthritic lesions in this model.

6N

CLINICAL AND BIOCHEMICAL CORRELATIONS IN OSTEOARTHRITIS(OA) OF THE HIP. R. Shuckett, Y.M. Goldberg, C.J. Malemud. Case Western Reserve University, University Hospitals of Cleveland, Cleveland.

Clinical and biochemical features were studied in 14 patients with primary hip OA undergoing total hip replacement(THR). Non-steroidal (NSAID) use, other joint OA and X-ray pattern of joint narrowing (classic superolateral versus medial(M) or concentric(C)) were assessed. Femoral head cartilage was set up in explant culture. Newly-synthesized (NS) PGs were measured by 20 hr $^{35}SO_4$ uptake and existing,endogenous (END) PGs by hexuronate content. Tissue extracts were eluted by associative chromatography into PG subpopulations based on hydrodynamic(hd) size. These PG subpopulations were resolved into PG subclasses in CsCl gradients. Paraffin sections were stained for metachromasy. Pearson Chi Square with Yates correction was used to compare variables.

Age above the median (> 63.5 yrs) correlated with bilateral hip OA and M or C narrowing ($p< 0.03$). Five patients had never received NSAIDs. Distribution of PGs into subpopulations or subclasses did not vary as a function of age, X-ray pattern, or NSAID use. For all samples, NS and END PGs eluted into 4 subpopulations on chromatography: 1 subpopulation of large PG aggregate(PG aggr), 1 with a hd size of PG monomer, and 2 additional subpopulations of smaller PG. Variations in this elution pattern were found, with a smaller hd size of PG aggr and a more distinct peak of small PG in some samples. These 2 features correlated with loss of peri-cellular metachromasy ($p=0.03$), suggesting differences in PG synthesis or processing. Elution patterns correlated strongly for NS and END PGs within a given sample ($p=0.007$) These observations suggest that OA cartilage explants synthesize PGs which are found in their existing extracellular matrix.

Clinical Utility and Specificity of Anticardiolipin Antibodies

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Abstract. **Established solid phase assays for anticardiolipin antibodies (aCL) are often characterized by high levels of nonspecific binding. As a result, only very high levels of aCL have been reported to be associated with a variety of clinical conditions including systemic lupus erythematosus (SLE), recurrent intravascular thrombosis and unexplained recurrent fetal loss. We have developed an ELISA replacing direct evaporation of soluble cardiolipin with cardiolipin micelles in physiological saline as the antigen binding step in the assay. Levels of IgG aCL were detected in various sera at dilutions of 1/100 to 1/3200, showing improved assay sensitivity. Assay specificity was determined using double stranded DNA and ovalbumin as irrelevant binding antigens and no crossreactivity was found. The controversial use of Tween 20 in the assay was investigated and results showed it decreases nonspecific binding without interfering in antibody detection. This assay has enabled us to identify differences in the prevalence and level of aCL antibodies in sera from healthy nonpregnant controls (0/25 positive), healthy pregnant controls (5/47 positive for IgG and 8/47 positive for IgM) and from women with unexplained recurrent fetal loss (16/62 and 14/62 positive, respectively). We support the observation that aCL are not normally distributed, and therefore nonparametric methods of statistical analysis are necessary to determine population prevalence. We confirm that aCL IgM are a relatively nonspecific finding, and extreme caution must be used in basing any clinical decisions on the presence of this antibody alone.** (*J Rheumatol 1991;***18:1849–55**)

Key Indexing Terms:
ANTICARDIOLIPIN ANTIBODIES
CARDIOLIPIN MICELLES
RECURRENT FETAL LOSS
ELISA

Anticardiolipin antibodies (aCL) have been associated with a variety of clinical conditions, including systemic lupus erythematosus (SLE), thrombosis, recurrent fetal loss, and thrombocytopenia[1-7]. In some reports, the predictive value of aCL has been determined and the decision to intervene therapeutically has been based upon their presence[8,9]. With the increasing number of documented clinical correlations, and decisions to prescribe corticosteroids and anticoagulants being based upon such detection, it is imperative that a uniform, sensitive and specific assay technique be employed so results from different groups can be compared and contrasted.

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We selected sera from women with unexplained recurrent fetal loss as the patient group to investigate for the prevalence and frequency of aCL. Because the decision to give an otherwise healthy woman immunosuppressive therapy during her pregnancy is so serious, it is essential that the basis for that decision be sound and incontrovertible. If the presence of aCL is the only indicator for such intervention, there must be confidence in the data that support the decision.

Since the introduction of the first specific assay for aCL, there has been controversy about their prevalence and clinical significance[1-6,10]. One of the major reasons for this ongoing debate is that a definitive assay system complete with descriptions of specificity and sensitivity has not been described, and normal values obtained by the different systems have not been subjected to uniform statistical analysis. We describe a new system, and show in detail the process by which we evaluated the previously published variables and developed the ELISA.

MATERIALS AND METHODS

Following standard nomenclature, we use aCL to represent antibodies to cardiolipin and aPL to represent the whole spectrum of antiphospholipid antibodies.

Preparation of cardiolipin micelles. Bovine heart cardiolipin (CL) (2 mg) in methanol (Sigma Chemical Co., St. Louis, Missouri) was transferred to a 10 ml conical glass tube. The CL was evaporated under a nitrogen gas stream while being continuously mixed using a vortex. After complete evaporation, the CL was redissolved in 0.5 ml of "wet" ether and mixed again. Physiological saline (NS) (pH 5.0) (0.5 ml) was added to the tube

dropwise during constant agitation. The ether in the milky solution was evaporated under a stream of N_2. The tube was placed in an ice bath, and the CL micelles were sonicated (10 μm vibrations) for 3 min (1-min intervals separated by 30-s rests). This preparation was finally diluted to 40 μg/ml in NS.

aCL ELISA. Ninety-six well, polystyrene, flat bottom microtiter plates (Nunc, Gibco, Burlington, Canada) were precoated with 100 μl/well 1% protamine sulphate for 1 h at room temperature (RT). After plates were washed with distilled water (dH_2O) 4 times, 100 μl/well of 40 μg/ml CL micellar solution was added and the plates were incubated overnight at 4°C. Immediately before use, the plates were washed as above and blocked with 2.5% heat inactivated, filtered horse serum (HS) (Gibco, Burlington, Canada) in normal saline (NS). Blocking was carried out for 75 min at RT. Plates were then washed with dH_2O containing 0.05% Tween 20 (Sigma Chemical Co., St. Louis, MO) and rinsed with dH_2O. Test and control sera were diluted 1/200 in 1% HS/NS/0.05% Tween 20 and added to triplicate wells. The plates were incubated for 75 min at RT. After washing, urease conjugated rabbit antihuman IgG or IgM (JD Biologicals, Mississauga, ON) was diluted to a predetermined concentration in 2.5% HS/NS/T before addition to the plates. They were again incubated at RT for 75 min. Urease substrate 620 (JD Biologicals), warmed to RT, pH 4.8, was added to the washed plates and the optical densities were read at 620 nm after 30 min incubation using a V-max Kinetic Microplate Reader (Molecular Devices Corporation, Palo Alto, CA).

Variation of antigen preparation. To determine the effect of antigen preparations on the quality of the assay results, 3 different methods testing 24 sera were compared. Each method used 96-well, polystyrene, flat bottom plates. (1) CL in solution in methanol was diluted to 40 μg/ml in NS and added to plates directly. (2) CL micelles were prepared as described above, diluted and added to the plates directly without using a precoat. (3) Plates were precoated with 1% protamine sulphate and CL micelles were then added. The assays were all run as described above.

Proteins used for blocking and in diluents. CL plates were prepared as described and various diluents were used for blocking, serum and enzyme dilutions. Fetal bovine serum (FBS) (HyClone Laboratories, Logan, UT), horse serum and normal human serum (NHS) were all heat inactivated and used for blocking the plates as follows: 2.5% HS/NS, 2.5% FBS/NS or 3% bovine serum albumen (BSA, fraction 5, Gibco Burlington, Canada) in NS were added to the wells and the plates were incubated at RT for 75 min. Twenty-four sera were serially diluted from 1/100 to 1/3200 in either 1% (v/v) HS/NS/T, 1% FBS/NS/T or 1% NHS/NS/T and added in triplicate to the wells for 75 min incubation at RT. Urease conjugated goat antihuman IgG or IgM was diluted in either 2.5% HS/NS/T, 2.5% FBS/NS/T or 3% BSA/NS/T and then added to washed plates which in turn were incubated at RT for 75 min. The remainder of the assays were run as above.

Enzyme conjugated antihuman immunoglobulin reagents. Plates were prepared and used as above except that 3 different enzyme conjugates were evaluated: urease, horse radish peroxidase (HRP) and alkaline phosphatase (Jackson Labs, BioCan, Mississauga, ON) (AP) conjugated antihuman IgG or IgM were serially diluted from 1/2000 to 1/32000 and each was tested with 24 known positive and negative sera. Appropriate substrates were prepared for each enzyme: urease substrate 620, pH 4.8, was warmed to RT; orthophenylene diamine (OPD, Sigma Chemical Co.) substrate (0.2%) in citrate buffer (pH 5.0) containing 0.015% H_2O_2 was held in the dark at RT until ready for use; 10 mg Sigma 104 phosphatase substrate (*p*-nitrophenyl phosphate disodium) was dissolved in 10 ml 0.1 M sodium barbital (pH 9.8) and then held in the dark at RT until ready for use. Optical density readings were taken every 15 min at the appropriate wavelengths for each substrate (urease: 620 nm; HRP: 450 nm; AP: 410 nm) for the first 2 h of substrate incubation.

Washing solution variations. To determine the affect of Tween 20, 5 plates were run, each with a variation in the washing solution. Tween 20 was introduced into the washing solution at incremental steps in the assay: plate 1: no tween used; plate 2: after the precoat; plate 3: after the CL coating step; plate 4: after the blocking step; and plate 5: after the serum incubation step.

Optical densities were recorded after 20 min incubation with substrate, and positive:negative ratios were calculated for each variation of the assay.

Specificity of the aCL assay. The specificity of the aCL assay was investigated by preincubations of test sera not only with irrelevant antigens, but also with other phospholipids. The following antigens were serially diluted from 10 μg/ml to 1 ng/ml in NS: cardiolipin, phosphatidylinositol (PI), phosphatidic acid (PA) and phosphatidyl serine (PS), all in micellar phase; dsDNA and ovalbumin. To each tube containing a concentration of one of the antigens or diluent only, was added 5 μl of a known positive or negative serum. The tubes were then vortexed, covered, and held at 4°C for 75 min. Samples were added to CL coated plates and assayed as outlined above.

The cross reactivity of serum anti-CL antibodies with other negatively charged phospholipids was determined by assaying sera with varying levels of anti-CL IgG and IgM for antibodies against PS, PI and PA. Micelles of each PL were prepared, bound to plates, then assayed as outlined above.

Prevalence of aCL in different clinical groups. To examine the clinical utility of this antibody, a target population of women with unexplained recurrent fetal loss was used. Sera were obtained from 25 healthy, nonpregnant volunteers, 47 healthy, pregnant women and from 62 women with unexplained recurrent fetal loss, all between the ages of 18 and 39. The healthy pregnant and nonpregnant women had unremarkable obstetrical and gynecological histories and no evidence of rheumatological disorder including the phospholipid syndrome when examined by a rheumatologist (CAL). The women with unexplained recurrent fetal loss had a history of 3 or more losses in the first trimester or 2 losses, with at least one loss occurring 14 weeks or later in gestation. In addition, none had evidence of anatomic, hormonal or genetic abnormalities that might account for the recurrent fetal losses. There was no significant difference between the mean ages in any group.

Sera from all subjects were serially diluted from 1/100 to 1/3200 and were assayed for aCL. Statistical significance of the data was determined using receiver operator characteristics (ROC) curve analysis to account for the nonparametric nature of the population distribution of this antibody.

RESULTS

Effect of antigen coating on aCL assay. Figure 1 shows the differences observed with variation in the preparation of CL used in the antigen coating step in the ELISA. The figure shows the results of 2 representative positive and negative specimens that clearly showed the differences between the antigen preparation steps. When detecting either aCL IgG or IgM, the use of protamine sulfate as a precoat and CL in micellar phase consistently resulted in a better differentiation between a positive and a negative result. Although the use of the protamine sulfate increased the background reading in the IgG assay, the increase in the OD of the positive specimens was far greater than the small increase in nonspecific binding. In addition, the plates bound with protamine sulfate and CL micelles were stable if held covered at 4°C for 2 weeks (data not shown).

Effect of different proteins used in blocking and in diluents. Figure 2 shows the results of 2 of the 24 sera tested in the IgG aCL assays using the various diluents. The IgM aCL assays gave similar results with the various blocking solutions used (data not shown). The use of 3% BSA/NS solution in the blocking step and in the enzyme dilution buffer led to unacceptably high background binding (solid lines, Figures 2A and B). However, the use of HS/NS as the dilu-

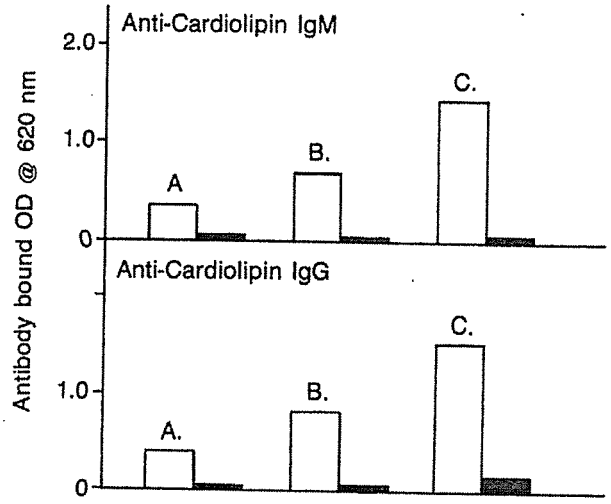

Fig. 1. Comparison of antigen binding techniques. Positive (clear bars) and negative (solid bars) sera were tested for aCL IgG and IgM using plates coated with (A) CL in chloroform/methanol evaporated directly onto the plate; (B) CL micelles in physiological saline bound to the plate during overnight incubation; (C) plate precoated with protamine sulfate, then CL micelles in physiological saline were bound to the plate during overnight incubation.

tion buffer decreased background binding even in the BS blocked plates (dotted lines, Figures 2A and B). Althou blocking with HS/NS decreased nonspecific backgrou binding, the presence of BSA in the enzyme dilution buff negated any benefit of such blocking (solid lines, Figur 2C and D). The critical step appeared to be the use of HS/I or NHS/NS in the serum and enzyme dilution buffers minimize nonspecific binding (dotted lines, Figures 2C a D). The use of FCS in either the blocking or dilution buffe led to more nonspecific binding than either HS/NS NHS/NS solutions but still less than the BSA (data n shown).

Comparison of enzyme conjugates. Three different enzyr conjugates were compared for sensitivity and specificity the aCL assay. Urease conjugates resulted in the greate assay sensitivity with least nonspecific binding activity (da not shown). The least effective conjugate in this micell ELISA was alkaline phosphatase which, although reacti at 30 min, required a 23 h incubation to achieve the positi OD results comparable with the urease conjugate after 1 min incubation. HRP reacted as efficiently as urease but h higher background binding and a lower positive reading f the positive specimens.

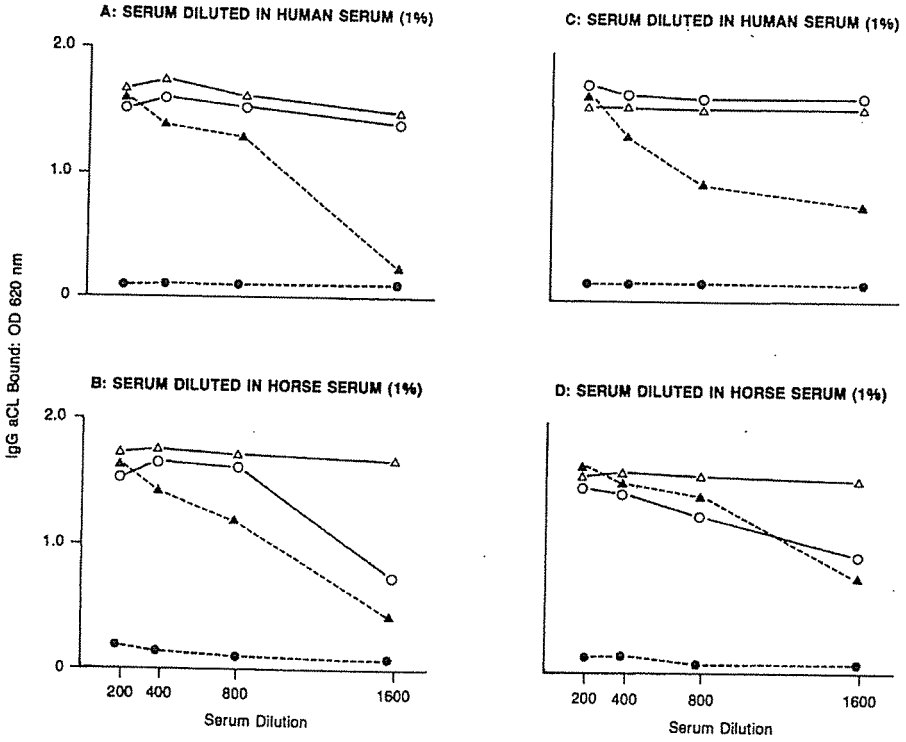

Fig. 2. Effect of different diluents used for blocking and enzyme steps in aCL IgG assay. Triangles represent a positive serum specimen; circles represent a negative serum specimen. Solid lines show results when enzyme is diluted in 3% BSA; dotted lines show results when enzyme is diluted in 2.5% horse serum. Figures 2A and B show results when BSA is used as a blocking agent; Figures 2C and D show results when HS is used as a blocking agent.

Tween 20 in the washing solution. Figure 3 shows a series of histograms representing positive and negative values of aCL IgG obtained when Tween 20 was introduced into the assay washing solution at different steps in the assay. In no case did the addition of Tween 20 decrease the assay sensitivity below that observed in the absence of Tween 20. In contrast, the binding of the negative specimen was decreased by the Tween 20. The results indicate that assay sensitivity was increased by the addition of Tween 20 to the washing buffer providing this occurred after the binding of CL antigen to the plates or after the blocking step. Institution of a Tween 20 supplemented washing buffer before either of these steps compromised assay sensitivity.

Specificity of the aCL assay. Both positive and negative sera were preadsorbed with 6 different antigens: CL, PI, PS, PA, dsDNA and ovalbumin. The results of an adsorbed positive serum are shown in Figure 4. As shown in Figure 4B, there was no crossreactivity between 3 other phospholipids (PI, PA and PS) and the aCL antibodies in this serum, because preadsorption did not decrease aCL IgG levels detected in the assay. Figure 4A shows the affects of preadsorption with dsDNA, ovalbumin and CL. No crossreactivity with either dsDNA or ovalbumin was found as indicated by their failure to inhibit aCL antibody binding. Only preadsorption with CL micelles reduced the binding of aCL IgG in this serum.

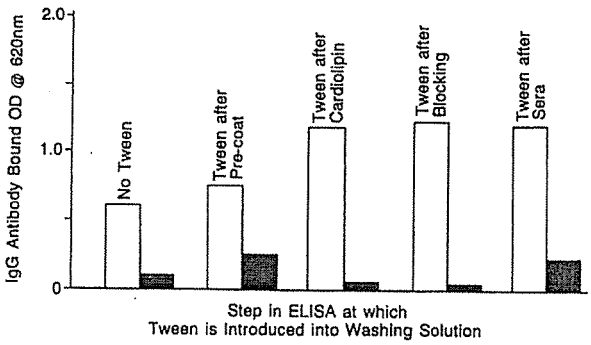

Fig. 3. Effect of Tween 20 on IgG aCL detected. Open bars represent positive sera; solid bars represent negative sera.

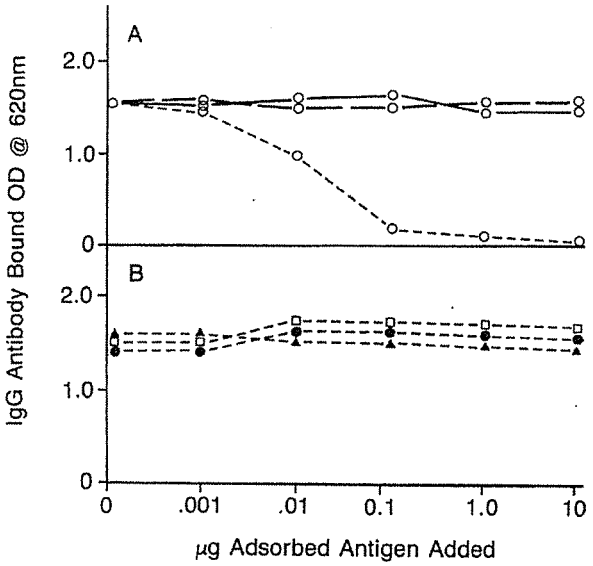

Fig. 4. Adsorption of aCL with different antigens. Figure 4A: serum adsorbed with dsDNA (———), ovalbumin (—— ——), and cardiolipin (--------). Figure 4B: serum adsorbed with PI (▲), PA (□) and PS (●).

aPL specificity. We tested a panel of sera not only for aCL but also for antibodies which bind to PS, PI and PA. Table 1 displays the results of this experiment. These data indicate that it is possible for an individual to have either antibodies directed against a variety of PL or perhaps polyspecific antibodies detecting a common binding site.

Occurrence of aCL antibodies in different reproductive groups. The upper limit of normal was calculated for each antibody class using ROC curve analysis[11]. Results for each group tested are shown in Figure 5. At a serum dilution of 1/200 (determined as the most discriminating dilution factor by the ROC analysis) no healthy women displayed aCL binding at an OD greater than 0.2. Healthy pregnant women had some IgM and IgG aCL antibodies (5/47 and 8/47, respectively) above that upper limit of normal. In the group with unexplained recurrent fetal loss, 16/62 (IgM) and 14/62 (IgG) were above the upper limit of normal.

Table 1. *Comparison of aPL antibodies detected in 7 different sera*

Antibody Detected		Patient 1	2	3	4	5	6	7
aPS	IgG	−	−	+	+	−	++	−
	IgM	+	−	++	+	+	+	−
aPI	IgG	−	−	+	−	−	−	−
	IgM	++	−	+++	−	+	−	−
aPA	IgG	−	++	−	+++	++	++++	+
	IgM	+++	+	+++	++	+++	+	−
aCL	IgG	−	+++	++	+++	++	++++	−
	IgM	+++	+	++++	+++	+	−	−

Symbols represent the level of antibody detected directed against each phospholipid (OD @ 620 nm).
− : OD < 0.200; + : 0.200 < OD < 0.500; ++ : 0.500 < OD < 1.000; +++ : 1.000 < OD < 1.500; ++++ : OD > 1.5000.

DISCUSSION

Since 1983 a number of different assays have been reported for the detection of aCL antibodies and due to variation in the methods and analyses, controversy still remains as to the prevalence and significance of this autoantibody[10]. We have attempted to clarify some of the problems by establishing a specific and sensitive ELISA and evaluating the various steps described by others.

CL micelles, used and recommended by at least 2 groups[7,8], were superior in differentiating antibody levels to the CL evaporated from methanol and/or chloroform solution which most assay systems use[7,8]. We found that plates coated with micelles are stable for 2 weeks at 4°C, an advantage not previously described.

At least one investigator attempted to overcome high background binding by subtracting nonspecific binding values (from plates not coated with antigen) from specific binding values (from plates coated with antigen)[4]. This system sometimes resulted in overall negative OD and the conclusion that the correction factor was "most significant for (but not limited to) sera with levels less than the mean plus 5 standard deviations for a normal population." Reports like these highlight the difficulty of optimizing the sensitivity and specificity of assays for aCL antibodies. Assays have been described using a variety of blocking solutions to minimize nonspecific binding. Harris, *et al* first used gelatin in their radioimmunoassay (RIA) but later discovered that both gelatin and BSA interfered with their results[10,12]. Even so, investigators are still using those blocking agents in their assays[7,12]. Manoussakis reported that whole adult animal serum appeared to give the best results (lowest backgrounds) and BSA had a detrimental effect when negative sera were tested[3]. Our results concur with those findings; we found HS to be superior as a blocking agent. Although NHS was as good, its availability, cost and possible infectious hazards make it a less desirable choice.

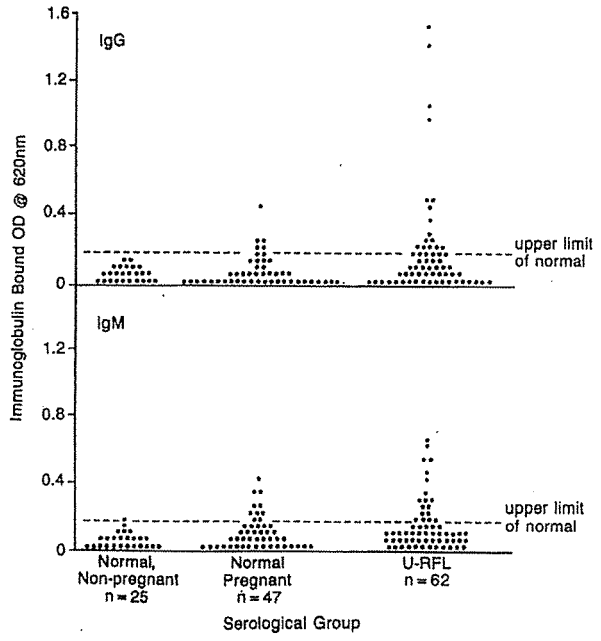

Fig. 5. Frequency of aCL IgG and IgM in 3 serological groups. Dots represent individual values in each category.

The use of an ELISA rather than an RIA has obvious safety advantages, and interestingly, the enzyme conjugate that we selected as the most appropriate is also the safest of the 3 conjugates we compared. Urease conjugates were best at differentiation of positive and negative sera and had a faster reaction time than the alkaline phosphatase conjugate used by most others[2,3,12-16]. The substrate for the urease conjugate is nontoxic in contrast to the substrates for both AP and HRP conjugates.

The use of Tween 20 in the washing and diluent solutions in aCL assays has been avoided in previously reported methods[17]. This may have been due to either negative or negligible effects observed with its addition in the first washing step directly after the CL is bound. Alternatively, Tween 20 may interfere with CL that has simply been evaporated onto a microtiter plate. Our investigations support the work of Cheng and Yap[18] and show that the addition of Tween 20 before the blocking step makes no difference to the assay results. However, its use after this step increases assay sensitivity considerably by reducing background or nonspecific binding, providing the antigen is bound to the plate in micellar form.

The crossreactivity of some aCL, aPL and anti-DNA antibodies has been widely described and is due to the shared phosphate backbone structures of the antigens[7,8,19]. We were able to identify sera with aCL IgG that did not crossreact with DNA and this has been noted by other investigators[6,7]. Some sera tested for antibodies against different PL were reactive with different combinations of negatively charged PL: these could represent either one polyspecific antibody or several monospecific antibodies. Experiments are currently underway to evaluate these hypotheses, but a discussion of the preliminary results is beyond the scope of this report.

The problem of determining an appropriate cutoff point for the upper limit of normal has been addressed by a number of statistical methods: raising the level to 3 or 5 SD above the mean in order to reduce to acceptable the number of normals with a positive value or using the percentile method to establish the OD above which only 2% of normals are positive[3-5,13]. Several investigators have observed that normal values for aCL antibodies are positively skewed and therefore nonparametric analysis should be used to establish a confidence interval[13,14,20]. We support the observation that the data are not normally distributed and find that ROC curve analysis provides a reliable cutoff level for both antibody classes[11]. Our aCL results are given as the number of sera

with OD > 0.200 at a dilution of 1/200. This represents not only the mean plus 2 SD of our normal population, but is also the best cutoff point suggested by ROC analysis. ROC analysis takes into account both the sensitivity and specificity of this antibody in differentiating healthy and patient populations and its nonparametric distribution. Based upon this method, we are confident that the 10.6% of our healthy pregnant women we found positive for IgG aCL is a representative and reliable figure. It is important to note the variations of positivity in the group with unexplained recurrent fetal loss and the healthy pregnant women. If we changed our upper limit of normal to 3 SD above the mean as Cowchock proposes[4], we would find 8.5% of our healthy controls and 29% of our group with unexplained recurrent fetal loss positive for aCL. If we changed our upper limit of normal to 5 SD as recommended by El Roiey, we would find 2.1% of our healthy women and 21% of our group with unexplained recurrent fetal loss positive. By altering our upper limit of normal, we find our control results more in line with published reports, but we have not altered the degree of positivity or increased the sensitivity of this antibody in differentiating the 2 groups. Between 19 and 21% more women in the group with unexplained recurrent fetal loss are positive for aCL than healthy pregnant women. We are proposing from the results of this study that more women with unexplained recurrent fetal loss have IgG aCL than women without a history of reproductive failure, and this is well illustrated, regardless of the cutoff point selected for this antibody.

Like others we find the levels of IgM aCL not significantly different regardless of clinical classification, and feel that it may indeed be a "relatively nonspecific finding"[22,23]. Indeed, Vaarala stated that it is important to realize that these antibodies occur very frequently in a wide variety of uncomplicated infections[14]. For example, although none of our nonpregnant control sera was positive for aCL, we found IgM aCL not only in women with unexplained recurrent fetal loss (14/62 or 22.6% IgM), but also in healthy pregnant women (8/47 or 17.0% IgM). Based upon our observations, this antibody could not be used as a sole entry criterion for therapeutic intervention in the hope of improving pregnancy outcome.

It has been reported that there is not only interlaboratory variation in aCL determination, but also day-to-day variation between results in the same laboratory[24]. Therefore, the decision to include a patient in an experimental therapeutic protocol based solely upon the presence of antibodies to CL becomes a difficult one. With such evidence available, it becomes obvious that extreme caution must be used in basing any clinical decisions on the presence of this antibody alone. We agree with Harris that the problem of evaluating the prevalence and significance of aCL antibodies will remain until all interested groups agree upon a standardized test with proven sensitivity and specificity[24]. The assay methodology we describe is novel in its use of CL micelles, urease conjugates, horse serum and Tween 20. It also offers the advantages of stability of prepared plates, speed of reaction time, low background binding, and negligible toxicity of reagents in comparison to previously described systems.

REFERENCES

1. Colaco CB, Male DK: Anti-phospholipid antibodies in syphilis and a thrombotic subset of SLE: distinct profiles of epitope specificity. *Clin Exp Immunol 1985;*59:449–56.
2. Cronin ME, Biswas RM, Vander-Straeton C, Fleisher TA, Klippel JH: IgG and IgM anticardiolipin antibodies in patients with lupus with anticardiolipin antibody associated clinical syndromes. *J Rheumatol 1988;*15:795–8.
3. Manoussakis MN, Gharavi AE, Drosos AA, Kitridou RC, Moutsopoulos HM: Anticardiolipin antibodies in unselected autoimmune rheumatic disease patients. *Clin Immunol Immunopathol 1987;*44:297–307.
4. Cowchock S, Fort J, Munoz S, Norberg R, Maddrey W: False positive ELISA tests for anticardiolipin antibodies in sera from patients with repeated abortions, rheumatologic disorders and primary biliary cirrhosis: correlation with elevated polyclonal IgM and implications for patients with repeated abortions. *Clin Exp Immunol 1988;*73:289–94.
5. Meyer O, Piette J-C, Bourgeous P, *et al:* Antiphospholipid antibodies: A disease marker in 25 patients with anti nuclear antibody negative systemic lupus erythematosus (SLE): Comparison with a group of 91 patients with antinuclear antibody positive SLE. *J Rheumatol 1987;*14:502–6.
6. Sturfelt J, Nived O, Norberg R, *et al:* Anticardiolipin antibodies in patients with SLE. *Arthritis Rheum 1987;*30:382–8.
7. Isenberg DA, Colaco CB, Dudeney C, Todd-Pokropek A, Snaith ML: The relationship of anti-DNA antibody idiotypes and anticardiolipin antibodies to disease activity in systemic lupus erythematosus. *Medicine 1986;*65:45–55.
8. Lafer EM, Rauch J, Andrzejewski JR, *et al:* Polyspecific monoclonal lupus autoantibodies reactive with both polynucleotides and phospholipids. *J Exp Med 1981;*153:897–909.
9. Hughes GRV, Harris EN, Gharavi AE, eds. Presentations from the second world symposium on anti-phospholipid antibodies. St. Thomas Hospital, London, 4th April, 1986.
10. Harris EN, Gharavi AE, Boey ML, *et al:* Anticardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. *Lancet 1983;*2:1211–4.
11. Bombardier C, Hawker J, Laskin CA, Raboud J, Soloninka CA: ROC analysis of autoantibodies in women with habitual abortion. (manuscript in preparation).
12. Unander AM, Norberg R, Hahn L, Arfors LA: Anticardiolipin antibodies and complement in ninety-nine women with habitual abortion. *Am J Obstet Gynecol 1987;*156:114–9.
13. Koskella P, Vaarala O, Makitalo R, Palosuo T, Aho K: Significance of false positive reactions and anti-cardiolipin antibodies in a nationwide series of pregnant women. *J Rheumatol 1988;*15:70–3.
14. Vaarala O, Palosuo T, Kleemola M, Aho K: Anticardiolipin response in acute infections. *Clin Immunol Immunopathol 1986;*41:8–15.
15. Sutjita M, Hohmann A, Raffaela C, Bradley J: Polyspecific human and murine antibodies to diphtheria and tetanus toxoids and phospholipids. *Clin Exp Immunol 1988;*73:191–7.

16. Harris EN, Gharavi AE, Patel SP, Hughes GRV: Evaluation of anticardiolipin test: report of an international workshop held on April 4, 1986. *Clin Exp Immunol 1987;*68:215–22.
17. Gharavi AE, Lockshin MD: Enhancement of anti-phospholipid antibody activity by Tween 20 (letter). *J Immunol Methods 1988;*114:277.
18. Cheng HM, Yap C: Enhancement of antiphospholipid antibody activity by Tween 20. *J Immunol Methods 1988;*109:253–5.
19. Valeseni G, Tincani A, Harris EN, *et al:* Use of monoclonal antibodies to identify shared idiotypes on anticardiolipin and anti-DNA antibodies in human sera. *Clin Exp Immunol 1987;*70:18–25.
20. Petri M, Rheinschmidt M, Whitting-O'Keefe Q, Hellman D, Corash L: The frequency of lupus anti-coagulant in SLE. *Ann Intern Med 1987;*107:524.
21. El-Roeiy A, Gleicher N: Definition of normal autoantibody levels in an apparently healthy population. *Obstet Gynecol 1988;*72:596–702.
22. Harris EN, Hughes GRV, Gharavi AE: Antiphospholipid antibodies: an elderly statesman dons new garments. *J Rheumatol 1987;*(suppl 13)14:208–13.
23. Harris EN, Gharavi AE, Hughes GRV: Antiphospholipid antibodies. *Clin Rheum Dis 1985;*11:591–609.
24. Harris EN: The second international anticardiolipin standardization workshop / the Kingston antiphospholipid antibody study (KAPS) group. *Am J Clin Pathol 1990;*94:476–84.

1478

ANTI-CARDIOLIPIN ANTIBODIES IN ACUTE HUMAN PARVOVIRUS B19 INFECTION. C.A. Soloninka, C.A. Laskin. University of Toronto, Toronto, ON, Canada.

Anti-cardiolipin antibodies (aCL) have been associated with a number of conditions including systemic lupus erythematosus, thrombosis, thrombocytopenia and recurrent fetal loss. aCL have also been reported to occur very frequently in conjunction with a number of uncomplicated infections and with HIV.

As part of an ongoing study regarding the effects of human parvovirus B19 infection, we examined 91 sera from patients with serological evidence of acute (IgM positive), convalescent (IgG positive) or no B19 infection for the presence of IgG aCL. Eighty-five percent of patients with acute B19 infection had circulating IgG aCL compared to 30.8% of convalescent sera and 6.5% of sera from those who have never had B19 infection. These differences were statistically significant (acute vs. never infected, $p<0.00001$; acute vs. convalescent, $p<0.05$). The levels of anti-B19 positivity were not correlated with the levels of IgG aCL ($r= -0.02$), indicating that the aCL are not just a result of polyclonal B-cell activation.

B19 infection has already been associated with anti-DNA and anti-lymphocyte antibodies and transient rheumatoid factor positivity. This is the first report of the production of aCL during B19 infection, and it confirms the development of a transient, subclinical autoimmune condition with B19. It also adds to the growing body of evidance that B19 infection should be ruled out when a patient presents with sudden onset symmetric polyarthritis and autoantibody positivity which might otherwise suggest a diagnosis of early RA or SLE.

1479

ANTIPHOSPHOLIPID ANTIBODIES FROM PATIENTS INFECTED WITH HUMAN B19 PARVOVIRUS HAVE SIMILAR SPECIFICITY AND CO-FACTOR DEPENDENCE TO THOSE FOUND IN SLE PATIENTS. Sozos Loizou, John K. Cazabon, Alex K.L. So, Derek Tait*, Mark J. Walport. Rheumatology Unit & Virology Dept.*, Royal Postgraduate Medical School, Du Cane Road, London W12 ONN, UK.

Raised levels of a number of autoantibodies characteristic of SLE, such as anti-dsDNA, anti-lymphocyte antibodies, aCL and ANA , as well as clinical manifestations such as Arthralgia, rash, thrombocytopenia, leucopenia, fetal wastage and hypocomplimentemia, have been described following human B19 parvovirus infection. We examined the spectrum of anti-phospholipid antibodies against, cardiolipin (aCL), phosphatidylserine, (aPS) and phosphatidylethanolamine, (aPE) in the sera of 12 patients following B19 parvovirus infection (Gp.I), 10 patients with other viral infections (Gp.II, two of each with CMV, Hep-A, EBV, VZV, Rubella), and in 15 syphillitic patients (Gp.III). For aCL, levels were raised in, 6/12(50%) of Gp.I, 2/10(20%) of Gp.II, and in 7/15(47%) of Gp.III patients; for aPS levels were raised in 10/12(83%), 6/10(60%), 8/15(53%), and for aPE levels were raised in 1/12(8%), 2/10(20%), and in 9/15(60%) patients respectively.

For the IgG isotype, a significant difference in, mean (±1 SEM) levels, was seen for aCL between Gp.I (20.29±7.88 units) and Gp.II (7.76±4.92, $z=-2.143$, $p<0.0162$), for aPS between Gp.I (13.96±3.5) and Gp.II (5.91±1.79, $z=-1.846$, $P<0.0329$) , and for aPE between levels in Gp.III (53.41±17.15), and both Gp.I (7.28±3.11, $z=3.8067$, $p<0.0001$), and Gp.III (5.74±2.07, $z=3.72$, $p<0.0001$) levels. When the effects of the aCL cofactor (β_2-GPI) on the binding of IgG aCl were compared between the 3 patient groups and to a group of 11 SLE patients, no significant difference was found in the number of patients showing enhanced binding, between the Gp.I (8/12, 66.7%) and SLE (6/11, 54.5%) patients, whereas this difference was significant between Gp.I and Gp.II (2/10, 20%) or Gp.III (1/11, 9.1%) patients respectively ($p<0.034$, $p<0.007$), as well as between SLE and Gp.III patients ($p<0.03$).

Our results indicate that aPL prevalence in B19 parvovirus infected patients is of similar specificity and cofactor dependence, to that reported for SLE but different from that found in syphilis and other viral infections.

ACR Abstract Session 67, Cytokines

Thursday, October 26, 1995, 10:00 AM–12:00 PM

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ENHANCED DEGRADATION OF ARTICULAR CARTILAGE IN FLARES OF ANTIGEN-INDUCED ARTHRITIS IN MICE: DIRECT EFFECT OF IL-1 ON CHONDROCYTES. Fons A.J. van de Loo, Onno J. Arntz, Peter LEM van Lent, Wim B. van den Berg. Department of Rheumatology, University hospital, 6525 GA Nijmegen, The Netherlands.

Between week 3 and 5 of a mild form of antigen-induced arthritis (AIA) the articular cartilage matrices are fully recovered from the arthritic insult. Injection of low amounts of the antigen (mBSA) into the affected knee joints caused a flare-up of the smouldering inflammation and a rapid and major loss of cartilage proteoglycans (PGs). Injection of neutralizing antibodies against TNFα (V1Q) 1 hour before flare-up induction had no effect whereas injection of anti-IL-1($\alpha+\beta$) antibodies markedly ameliorated the flare-up related cartilage destruction. The latter was validated by treatment of mice with human IL-1 receptor antagonist. IL-1 was clearly demonstrated in wash-outs of joints using a bioassay and immunohisto-chemically on whole knee joint sections taken between 1-6 hours of the flare-up. Injection of IL-1 mimicked the antigen-induced flare-up reaction. IL-1 caused a 30-50% enhanced breakdown of $^{35}SO_4$-prelabeled PGs, 24 hours after i.a. injection and this was clearly visible on safranin-O stained knee-joint sections. IL-1 injected into naive joints resulted in 10-15% loss of PG which could not be seen by histology. Treatment of mice with anti-5C6 antibodies prevented the integrin mediated influx of granulocytes but injection of IL-1 still caused cartilage depletion. Additionally, *in vitro* challenge of arthritic repair cartilage with IL-1 resulted in enhanced PG breakdown (19%) compared to cartilage of the normal contralateral joint. For this, IL-1 plays a pivotal role in the antigen-induced flare-up of chronic arthritis and cartilage is more prone for IL-1 effects in the repair phase.

1481

EXPRESSION OF A SOLUBLE INTERLEUKIN-1 RECEPTOR CDNA IN RABBIT SYNOVIAL CELL-LINE (HIG-82) IN VITRO RESULTS IN BLOCKADE OF INTERLEUKIN-1 STIMULATED STROMELYSIN MRNA UP-REGULATION.

Fuad Mehraban, Sriram Kasturi. Case Western Reserve University, Department of Medicine, 10900 Euclid Avenue, Cleveland OH 44106.

Introduction: Interleukin-1 (IL-1) is involved in the up-regulation of matrix metalloproteases (MMP) in cartilage and synovium. These enzymes are involved in cartilage degradation in rheumatoid arthritis (RA) and osteoarthritis (OA). In this study we tested the possibility of blocking IL-1-stimulated MMP expression by insertion into a rabbit synovial cell-line (HIG-82) of an expression vector containing a synthetic cDNA encoding the extracellular domain of the human chondrocyte type-1 IL-1 receptor (IL-1R).

Methods: A 1 Kb cDNA encoding the ligand-binding domain of human chondrocyte type-1 IL-1R was synthesized using the polymerase chain reaction (PCR). PCR primers were designed such that the amplified sequence contained the translational start codon, and a stop codon before the transmembrane domain sequence. The resultant cDNA was cloned, sequenced, inserted directionally into an expression vector, and transfected into HIG-82 cells. Control cells were transfected with vector only. Medium from stable transfectants was tested for soluble IL-1R (sIL-1R) using a ^{125}I-IL-1β binding assay. Confluent culture of a clone producing sIL-1R was stimulated with IL-1β (0, 0.2,1 ng/ml). RNA was made, Northern blotted and probed with ^{32}P-cDNA for rabbit stromelysin.

Results: Expression of IL-1R extracellular domain cDNA in synovial cells resulted in appearance of ^{125}I-IL-1β binding in the medium indicative of secretion of sIL-1R. Cells transfected with vector alone did not produce sIL-1R . Expression of sIL-1R by synovial cells in vitro abrogated both basal and IL-1β stimulated stromelysin mRNA expression. HIG-82 cells or cells transfected with empty vector expressed stromelysin mRNA.

Conclusion: Genetic modification of joint tissue resulting in expressionof sIL-1R blocks IL-1-stimulated MMP expression, and may be beneficial as a therapeutic modality in amelioration of IL-1- mediated cartilage degradation in arthritis.

Supported by NIH grant AR-20618.

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Interleukin-1β Converting Enzyme Inhibition Blocks Progression of Type II Collagen-Induced Arthritis in Mice. Matthew W. Harding, George Ku, Ted Faust, Linda L. Lauffer and David J. Livingston. Vertex Pharmaceuticals Incorporated, 40 Allston Street, Cambridge, MA 02139

IL-1β is a principal mediator in the pathogenesis of inflammatory disease. The IL-1β-converting enzyme (ICE) is required for processing of the 31 kDa IL-1β precursor to generate proinflammatory mature 17 kDa IL-1β. ICE inhibitors may therefore have therapeutic benefit in rheumatoid arthritis, osteoarthritis and other inflammatory diseases. We investigated the effect of two irreversible peptidyl ICE inhibitors, VE-13,045 and VE-16,084, on IL-1 production *in vitro* and *in vivo* in acute and chronic inflammatory disease models. *In vitro*, VE-13,045 and VE-16,084 inhibited IL-1β secretion by LPS-stimulated human adherent mononuclear cells (IC_{50}'s of 0.4 μM and 2.0 μM, respectively) and murine splenic monocytes (IC_{50}'s of 10 μM and 1.3 μM, respectively). VE-13,045 and VE-16,084 also inhibited LPS stimulated IL-1α secretion, although with reduced potency. *In vivo*, a single intraperitoneal dose of VE-13,045 (50 mg/kg) administered to mice 30-75 minutes after LPS (40 mg/kg) reduced IL-1β and IL-1α serum levels by 25-80%. In a mouse model of Type II collagen-induced arthritis (CIA), prophylactic treatment with VE-13,045 (50 and 100 mg/kg/day) significantly delayed the onset of inflammation, with a 60% overall reduction in disease severity. VE-13,045 was more effective than either indo-methacin (2 mg/kg/day) or methyl prednisolone (10 mg/kg/day). VE-13,045 was also effective in reducing inflammation and progression of arthritis when administered to mice with established CIA. Histological analysis of wrist joints shows a reduction in synovial membrane damage, inflammatory cell infiltration and fibrosis, and cartilage erosion in VE-13,045-treated animals. The efficacy of an ICE inhibitor in CIA suggests that ICE is an import target for design of novel anti-inflammatory or disease modifying anti-rheumatic drugs.

1483

MODULATION OF PROINFLAMMATORY CYTOKINE RELEASE IN RHEUMATOID SYNOVIAL MEMBRANE CELL CULTURES WITH AN ANTI TNFα MONOCLONAL: COMPARISON WITH BLOCKADE OF IL-1 USING THE RECOMBINANT IL-1 RECEPTOR ANTAGONIST. Fionula M. Brennan, Debra M. Butler, Ravinder N. Maini and Marc Feldmann. Kennedy Institute of Rheumatology, Sunley Division, Hammersmith London, W6 8LW, UK.

Although there is an extensive literature on cytokine regulation using human cell lines *in vitro*, little is known about cytokine regulation within inflammatory tissue *in vivo*. Previously, we demonstrated that in rheumatoid synovial membrane cultures, a complex, but pathophysiological relevant mixture of cells, the addition of a neutralising anti-TNFα antibody inhibited the production of IL-1 and GM-CSF, indicating the presence of a cytokine 'cascade' in this inflammatory tissue.

In this report we were interested to determine whether this cytokine cascade in rheumatoid synovial tissue *also* extended to IL-6 and IL-8. Thus the effect of blocking endogenous TNF bioactivity on cytokine production in dissociated RA synovial membrane cells (n=10) was determined using the chimeric anti-TNFα antibody (cA_2) at 2.5μg/ml and compared with the effects of blocking endogenous IL-1 activity using the the IL-1 antagonist, (IL-1ra) at 100ng/ml. We confirmed that blockade of TNF activity inhibited IL-1 production ($p<0.022$ at day 5) but that within the IL-1 family it was IL-1β in particular which is down regulated. Secondly we demonstrated that the cytokine interactions are unidirectional, in that neutralisation of TNFα blocked IL-1β production, whereas TNFα production was not modulated by the IL-1 receptor antagonist (IL-1ra). The production of either IL-6 or IL-8 produced in nanogram amounts throughout the culture period were downregulated by the neutralisation of either TNFα *or* IL-1. Thus by day five, IL-6 production was reduced from 761 ± 545 ng/ml to 462 ± 365 ng/ml with cA_2 ($p<0.008$) and to 461 ± 297ng/ml with IL-1ra ($p<0.019$). Further additive effects were observed when both antagonists were combined ($p<0.008$). Similarly for IL-8, control cultures levels were reduced from 840 ± 610 ng/ml to 510 ± 370 ng/ml with cA_2 ($p<0.006$), and to 623 ± 506 ng/ml with IL-1ra ($p<0.041$). Both antagonists together further reduced IL-8 levels ($p<0.006$). These results suggest a mechanism for the profound anti-inflammatory effects and consequent clinical benefit noted recently in a clinical trial of RA patients using a chimeric anti-TNFα antibody.

ACR MINISYMPOSIUM III
Gateway 102-3, Moscone 10:00 am - 11:45 pm

Moderator: Stanley J. Naides, MD, Iowa City, IA
Vincent Agnello, MD, Burlington, MA

VIRUSES AS CAUSES OF RHEUMATIC DISEASES

10:00 **Introduction:** Stanley J. Naides, MD. University of Iowa, Iowa City, IA.

10:30 **1475. The role of hepatitis C virus in cutaneous vasculitic lesions in patients with type II mixed cryoglobulinemia.** V Agnello. Burlington and Bedford, MA.

10:45 **1476. A prospective case-controlled study of the prevalence of hepatitis C virus-related cryoglobulinemia at an inner city hospital.** G Kerr. Washington, DC.

11:00 **1477. Persistent infection of human parvovirus B19 and arthritis.** T Sasaki. Sendai, Japan.

11:15 **1478. Anti-cardiolipin antibodies in acute human parvovirus B19 infection.** CA Soloninka. Toronto, Canada.

11:30 **1479. Antiphospholipid antibodies from patients infected with human B19 parvovirus have similar specificity and co-factor dependence to those found in SLE patients.** S Loizou. London, United Kingdom.

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ACR ABSTRACT SESSION 67
Gateway 104, Moscone 10:00 am - 12:00 pm

Moderators: William P. Arend, MD, Denver, CO
Gary S. Firestein, MD, San Diego, CA

CYTOKINES

10:00 **1480. Enhanced degradation of articular cartilage in flares of antigen-induced arthritis in mice: direct effect of IL-1 on chondrocytes.** FAJ van de Loo, OJ Arntz, PLEM van Lent and WB van den Berg. Nijmegen, The Netherlands.

10:15 **1481. Expression of a soluble interleukin-1 receptor cDNA in rabbit synovial cell-line (HIG-82) *in vitro* results in blockade of interleukin-1 stimulated stromelysin mRNA upregulation.** F Mehraban and S Kasturi. Cleveland, OH.

10:30 **1482. Interleukin-1β converting enzyme inhibition blocks progression of type II collagen-induced arthritis in mice.** MW Harding, G Ku, T Faust, LL Lauffer and DJ Livingston. Cambridge, MA.

10:45 **1483. Modulation of proinflammatory cytokine release in rheumatoid synovial membrane cell cultures with an anti-TNFα monoclonal: comparison with blockade of IL-1 using the recombinant IL-1 receptor antagonist.** FM Brennan, DM Butler, RN Maini and M Feldmann. London, UK.

11:00 **1484. Potential clinical relevance of the mechanism of inhibition of immune function by the mouse/human chimeric anti-TNFα antibody, cA2.** MR Dalesandro, CS Kinney, B Frederick, RE Jordan and J Ghrayeb. Malvern, PA.

11:15 **1485. Inhibition of NF-κB nuclear translocation and TNF-α gene expression by a novel adenosine A_3 receptor agonist.** TL Bowlin, CD McWhinney, DR Borcherding, CK Edwards, PF Hoffman, L Watts and JA Wolos. Cincinnati, OH.

11:30 **1486. Effect of IL-10 on murine SCW arthritis: role of TNF and IL-1 in joint inflammation and cartilage destruction.** LAB Joosten, MA Helsen and WB van den Berg. Nijmegen, The Netherlands.

11:45 **1487. Variable transgenic expression of tissue inhibitor of metalloproteases-1 by synoviocytes and chondrocytes *in vitro*.** D Vallance and B Roessler. Ann Arbor, MI.

1419

ANTI-CARDIOLIPIN IgG IN WOMEN WITH RECURRENT FETAL LOSS. C.A. Soloninka, C.A. Laskin, C. Bombardier, D. Wong, K.A. Spitzer, L.J. Fielding. University of Toronto, Toronto, ON, Canada.

Women with systemic lupus erythematosus (SLE) frequently experience recurrent fetal loss (RFL), which has been repeatedly associated with the presence of anticardiolipin antibodies (aCL). It has been proposed that aCL or a circulating anticoagulant may be a marker or even a causative agent for RFL in women without SLE. As a result, many clinicians are routinely screening for this antibody and treating prophylactically with prednisone or heparin and/or aspirin on the assumptions that aCL is both strongly prevalent and a significant risk factor in this population of women with RFL. However, our investigations do not support these assumptions.

We evaluated 1185 women (700 with unexplained RFL, and 485 controls) for the presence of IgG aCL, using 3 different assay systems over 9 years. Only 3.9% of the RFL population and 4.5% of the controls were positive for IgG aCL, giving a sensitivity for this antibody of 0.39, and a specificity of 0.95.

We then surveyed 185 publications in a literature review, and selected for comparison only those which reported appropriately standardized aCL IgG results in identically documented women with unexplained RFL. We found 14 additional studies which, including ours, totalled 2702 women with U-RFL. The IgG aCL was positive in 147 patients, a population prevalence of 5.4%. The sample sizes ranged from 35 to 700, and the IgG aCL prevalences in the 14 studies ranged from 0 to 17.1% using both in-house assays and commercial kits.

Based upon our own observations with our unselected controls and our large sample of women with U-RFL, and supported by a thorough review of the literature, we propose that the prevalence of this antibody in women of childbearing age is too low to justify both the time and expense of routine screening in a general obstetric population. Furthermore, there is scant evidence to support prophylactic treatment of those few normal pregnant women who do have circulating IgG aCL.

1420

HUMAN MONOCLONAL ANTICARDIOLIPIN ANTIBODIES REACT IN VITRO WITH ENDOTHELIAL CELLS THROUHG THE ADHERED BETA 2 GLYCOPROTEIN I AND INDUCE ENDOTHELIAL ACTIVATION. Del Papa N, Guidali L, *Sala A, *Folco GC, *Khamashta MA, [b]Ichikawa K, [b]Koike T, *Hughes GRV, ^Balestrieri G, ^Tincani A, Meroni PL. Department of Internal Medicine and *Pharmacology - University of Milan, *The Rayne Institute, St Thomas' Hospital London, ^Clinical Immunology - Spedali Civili, Brescia, [b]Department of Medicine II, Hokkaido University, Sapporo, Japan.

Objective. To investigate whether human monoclonal anti-cardiolipin antibodies recognising cryptic epitope on beta 2 glycoprotein I (β2GPI) are able to react with endothelial cells in vitro, whether β2GPI plays any role in this reactivity and finally whether the antibody binding can affect endothelial function.

Methods. Three human monoclonal antibodies from patients with the antiphospholipid syndrome (APS), two displaying a binding against cardiolipin and γ irradiated β2GPI-coated plates and one without any reactivity, have been studied for: a)their binding to endothelial cell cultured with and without bovine or human β2GPI. b) their ability to induce or upregulate cell surface expresion of E-selectin, ICAM-1 and VCAM-1, c) their ability to stimulate the secretion of pro-inflammatory cytokines such as Interleukin-6 (IL-6) and d) their capability to affect prostacyclin metabolism measured as 6 Keto-PGF 1 α levels in the supernatants.

Results. The two monoclonals positive for anti-cardiolipin and anti- β2GPI activity displayed a clear endothelial cell binding which declined on endothelial cells incubated in serum-free medium and it was restored after addition of exogenous human β2GPI in a dose dependent manner. The antibody binding induced the ex-novo expression of E-selectin and VCAM-1 and up-regulated the presence of ICAM-1 molecules; the incubation of endothelial monolayers with the antibodies significantly increased the IL-6 and 6-keto-PGF 1α levels in the supernatants. The control monoclonal did not display any endothelial reactivity and did not affect the functional parameters investigated.

Conclusions. Human monoclonal antibodies from APS patients able to react with cryptic epitope on β2GPI can recognize the plasma cofactor adhered on the endothelial cell surface. Their binding induces an endothelial activation in vitro as demonstrated by the upregulated adhesion molecule expression and by increased arachidonic acid metabolism and secretion of IL-6. Altogether these findings support the hypothesis that an endothelial cell surface binding leading to modulation of endothelial function can represent one of the potential in vivo pathogenetic mechanism for anti-phospholipid antibodies.

1421

EXPRESSION AND SITE-DIRECTED MUTAGENESIS OF HUMAN β_2-GLYCOPROTEIN I: IDENTIFICATION OF THE MAJOR PHOSPHOLIPID BINDING SITE. Yonghua Sheng, Steven Krilis, Department of Medicine, University of New South Wales, The St. George Hospital, Kogarah, NSW 2217, Australia, Andrej Šali, Martin Karplus, Department of Chemistry, Harvard University, Cambridge, Massachusetts 02138, USA.

β_2-Glycoprotein I (β_2-GPI), a serum protein with anticoagulant properties, plays a vital role in the binding of the anticardiolipin (aCL) antibodies purified from autoimmmune disease to cardiolipin (CL). A highly positively - charged amino acid sequence, Lys282 - Asn - Lys - Glu - Lys - Lys287, located in the fifth domain of β_2-GPI, has been predicted to be exposed on the surface of β_2-GPI and to be the phospholipid binding site. In order to determine the role of these residues in the binding of β_2-GPI to phospholipid, we altered a single amino acid Lys286 as well as different combinations of Lys284, Lys286, and Lys287 to Glu by site-directed mutagenesis and expressed the wild type and mutant cDNAs in a baculovirus system. A single amino acid change from Lys286 to Glu resulted in 50% reduction in both the cofactor activity of β_2-GPI for aCL antibodies binding to CL and in the inhibition of binding of iodinated native β_2-GPI to CL activity compared to wild type β_2-GPI. However the mutant2k (from Lys286, Lys287 to Glu286, Glu287), mutant2k$_a$ (from Lys284, Lys287 to Glu284, Glu287) and mutant3k (from Lys284, Lys286, Lys287 to Glu284, Glu286, Glu287) possessed no cofactor activity for binding of aCL antibodies to CL as well as no inhibitory activity on the binding of iodinated native β_2-GPI to CL. These results indicate that the residues Lys284, Lys286 and Lys287 are critical for β_2-GPI binding to anionic phospholipids.

1422

UPREGULATED EXPRESSION OF INDUCIBLE NITRIC OXIDE SYNTHASE IN SLE: EVIDENCE FOR ACTIVATED ENDOTHELIUM. H. Michael Belmont, David Levartovsky, Ashok R. Amin, Mary Louise Skovron, Jill Buyon, Ralph Giorno, John Rediske, Steven B. Abramson. Hospital for Joint Diseases/NYU Medical Center New York, N.Y. 10003; Res. Dept., Pharma. Div., CIBA Corp. Summit, N.J. 07901.

Nitric oxide (NO) has been implicated in the pathogenesis of a SLE-like illness in MRL-lpr/lpr mice. Therefore, we examined NO production in a cross-sectional study of 46 SLE patients. Serum nitrates/nitrites (NOx), the stable metabolites of NO, were measured via the Greiss reaction. Serum NOx levels were significantly elevated (37.4 ± 5.5 uM) compared to healthy controls (15.4 ± 6.9 uM). Non-parametric analysis revealed a significant ($p < .01$) correlation between NOx and DNA ($r=0.38$). The correlation coefficient was 0.50 ($p=.04$) between NOx and SLEDAI. NOx levels were significantly greater in patients with active SLE (SLEDAI > 5, n=9, 46.2 ± 21.3 uM) versus inactive SLE (SLEDAI < 5, n=10, 29.9 ± 20.7 uM). In order to identify the cellular source of NO production we obtained biopsies of nonlesional, non-sunexposed skin from 8 SLE patients. NO synthase (NOS) expression was determined immunohistochemically using specific monoclonal antibodies to inducible nitric oxide synthase (iNOS) or to constitutive endothelial cell NOS (ecNOS). Expression of ecNOS was not different for active versus inactive SLE patients or controls. In contrast, there was markedly increased endothelial cell (EC) expression of iNOS in SLE patients with active (n=5) versus inactive (n=3) disease or controls (n=4). This is consistent with our previous report that SLE disease activity is associated with increased EC expression of three distinct adhesion molecules, ICAM-1, E-selectin, and VCAM-1 (A&R 37:376-83,1994). These data indicate that NO, a recognized inflammatory mediator, is produced in excess in SLE patients and identifies an upregulated iNOS isoform, induced in EC, as a potential source. This latter intriguing finding supports the hypothesis that cytokine (and/or complement) activated EC play a central role in the development of vascular injury that characterizes SLE.

1423

TREATMENT OF IMMUNE-COMPLEX MEDIATED GLOMERULONEPHRITIS WITH C5 SPECIFIC MONOCLONAL ANTIBODY. Yi Wang, Qila Hu, Scott Rollins, Joe Madri†, and Louis Matis, Alexion Pharmaceutical Inc., 25 Science Park, New Haven, CT 06511, †Department of Pathology, Yale University School of Medicine, New Haven, CT 06510

Activated components of the complement system are potent mediators of inflammation that may play an important role in numerous immune-complex mediated autoimmune diseases, including glomerulonephritis in patients with systemic lupus erythematosus. To evaluate the role of complement activation in immune-complex mediated glomerular inflammation, we have utilized monoclonal antibodies (mAbs) that inhibit the complement cascade at C5. These mAbs block the generation of the major chemotactic and proinflammatory factors C5a and C5b-9, but preserve the critical C3b-mediated immunoprotective and immunoregulatory functions. In this study, we demonstrate the efficacy of C5 inhibition in the treatment of lupus nephritis in NZB/NZW F1 mice and a rapid progressive nephritis (RPGN) model induced in B10D2/nSn mice by repeatedly immunization with heterologous protein. Systemic administration of the anti-C5 mAbs effectively inhibited membrane attack complex (C5b-9) formation in vivo and significantly ameliorated immune complex mediated glomerulonephritis in both models, while animals treated with control mAbs developed renal failure characterized by persistent proteinuria and elevated serum BUN levels. Histological examination revealed that anti-C5 treatment effectively inhibited the formation of epithelial crescents and the recruitment of $CD11b^+$ inflammatory cells into glomeruli. In contrast to immune-complex mediated glomerular inflammation, the cutaneous immune-complex mediated Arthus reaction was only marginally inhibited in the same individuals treated with the anti-C5 mAbs at a dose sufficient to successfully block RPGN. These findings suggests that the predominant pathway by which an immune-complex initiates inflammation may depend on several factors, such as the tissue site of immune complex deposition. Our results provide persuasive evidence that C5-specific mAb therapy is an effective approach for the treatment of various immune-complex mediated glomerulonephritides and demonstrate a critical role for activated terminal complement components in immune-complex mediated tissue inflammation.

ACR Abstract Session 54, Clinical Aspects of Vasculitis
Wednesday, October 25, 1995, 2:15 PM–3:45 PM

1424

CLINICAL FEATURES AND THERAPEUTIC MANAGEMENT OF SUBGLOTTIC STENOSIS IN PATIENTS WITH WEGENER'S GRANULOMATOSIS. CA Langford, MC Sneller, CW Hallahan, GS Hoffman, WA Kammerer, C Talar-Williams, AS Fauci, RS Lebovics. National Institutes of Health, Bethesda, MD 20892.

Subglottic stenosis (SGS) is a potentially life threatening lesion that occurs in approximately 20% of patients with Wegener's granulomatosis (WG). We have followed 43 patients with SGS at the NIH and report on their clinical characteristics and management.

The diagnosis of SGS was made a median of 12 months after the onset of symptoms of WG. Six of the 43 patients (14%) had disease limited to the upper respiratory tract; the remaining 37 (86%) also had involvement of 1 or more major organ systems. Only 22 of 43 (51%) patients had active disease outside the upper respiratory tract at the time SGS was diagnosed. Twenty-one of 43 (49%) patients were receiving immunosuppressive therapy at the time their SGS was diagnosed and 18 of 43 (42%) patients required tracheostomy. Ten of these 18 patients (56%) required a tracheostomy despite receiving at least 2 months of systemic immunosuppressive therapy.

Since 1991 we have treated 20 patients with SGS using an intratracheal glucocorticoid injection and dilation procedure. Thirteen of these 20 patients required systemic immunosuppressive therapy for active disease outside the upper respiratory tract. The remaining 7 patients had isolated SGS and were treated only with intratracheal glucocorticoid injection and dilation. Once beginning this local treatment, none of the 20 patients required a tracheostomy and 6 tracheostomies placed prior to local treatment were removed. In 113 procedures only 1 complication has occurred.

SGS is a manifestation of WG that can occur independently of other features of active disease and is frequently unresponsive to systemic immunosuppressive therapy. SGS can be optimally managed using intratracheal injection-dilation treatment and, in the absence of other manifestations of active WG, does not require systemic immunosuppressive therapy.

1636

CIRCULATING AUTOANTIBODIES IN WOMEN WITH UNEXPLAINED RECURRENT PREGNANCY LOJSES: RESULTS FROM AN EVALUATION OF 783 WOMEN.
C.A. Laskin, K.A. Spitzer, C.A. Soloninka, C. Bombardier. University of Toronto, Toronto, Ontario, Canada.

Utilizing a large population, we determined the expected prevalence of circulating autoantibodies including, but not limited to, the lupus anticoagulant (LAC) and anti-cardiolipin IgG (αCL) in unexplained recurrent pregnancy loss.

As part of a randomized clinical trial, we screened 783 women with 2 or more pregnancy losses unexplained by hormonal, genetic, anatomic or clinical abnormalities for the following autoantibodies: ANA, anti-ss- and anti-dsDNA (IgG and IgM), anti-lymphocyte IgM, LAC and αCL.

385/783 women (49%) had at least one circulating autoantibody. The LAC or αCL was positive in 136 (25%) of the sample although the LAC was far more prevalent (Table). Other autoantibodies were found in 24% of our population. None of the women displayed any clinical manifestation of a connective tissue disease.

Autoantibody	% pos (n=783)
ANA	20.7
Lymphocyte	15.2
ss DNA	8.1
ds DNA	7.6
LAC	21.2
αCL IgG	3.9

Based upon these results, we conclude that the LAC and αCL are two markers indicating an immune pathogenesis in women with unexplained recurrent pregnancy losses. The immune evaluation of unexplained recurrent pregnancy loss must therefore include a comprehensive autoantibody panel to ensure identification of all women who might benefit from therapeutic intervention.

1637

PREGNANCY OUTCOME IN SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) AND ANTIPHOSPHOLIPID SYNDROME (APS). Azucena Ramos, Guadalupe Veloz, Juan Garcia, Leonor Barile, Juan M. Miranda, Luis J Jara.Rheumatology and Obstetrics Departments. Centro Médico La Raza, IMSS, México City,02990. México.

Therapy for the prevention of fetal loss in both SLE and APS remains controversial. It has become well established that the prevention of fetal loss is not a hundred percent effective. In addition, current therapeutic protocols have a high morbility rate.

Objective: To assess the maternal and fetal outcomes in SLE and APS patients.

Patients and methods: We prospectively studied 21 pregnancies in 8 SLE patients and 13 APS (5 primary and 8 secondary to SLE). Patients were seen in a multi-disciplinary clinic by rheumatologists and obstetricians each month. Treatment : Group I. SLE patients, aCL negative: prednisone 10 mg plus low-dose aspirin. Lupus flares were treated by increasing prednisone dose. Group II. 2° and 1°APS with history of at least one fetal loss: Prednisone 40 mg/d for 4 weeks, the dose was then tappered 10 mg every 4 weeks, up to a maintenance dose of 5 mg/d; 5,000 units of unfractionated heparin subcutaneously each 12 hours were added after the first month of prednisone, and low-dose aspirin was used throughout the pregnancy. Results: The live birth rate increased from 83% to 87% in group I, and from 50% to 92% in group II. A high incidence of maternal and fetal complications was observed: prematurity (38%), fetal distress (44%), low birth weight (61%), lupus flares (30%), hypertension (16%), preterm membranes rupture (11%), oligohydramnios (45%), and heparin-related injection site bruises (46%). Vertebral fractures were not found. We studied 9 placentas: 2 in Group I, and 7 in Group II. All placentas had low weight (< 450 gr), infarcts and calcifications. However, Group II placentas, exhibited more extensive infarcts accompanied by severe angiosclerosis.

Conclusion: Both treatment modalities improved the live birth rates in our patients, in spite of a high incidence of complications and persistent placental abnormalities.

1638

SYSTEMIC LUPUS ERYTHEMATOSUS AND PREGNANCY IN GREEK PATIENTS.
Panagiotis E. Georgiou, Eudokia N. Politi, Vayia Sakka, Alexandros A. Drosos, Department of Internal Medicine, Medical School, University of Ioannina, Greece.

Objective: To investigate the reciprocal relation between pregnancy and systemic lupus erythematosus (SLE).

Patients: Forty-seven pregnant SLE patients (mean age 24.3±2.5 years) with 59 pregnancies, 54 non pregnant SLE patients (mean age 31.9±7.7) and 70 healthy pregnant controls (mean age 27.2±6.2) with 70 pregnancies were included in this study. All pregnant SLE patients were in remission at the onset of pregnancy and were treated with prednisone (≤ 10 mg/day), hydroxychloroquine (200 mg/day) or azathioprine (100 mg/day).

Results: A) Effects of SLE on pregnancy: There were no differences regarding prematurity, or fetal loss between pregnant SLE patients and control group. Of the 59 SLE pregnancies, 36 (61%) were delivered at term, 3 (5%) were premature deliveries, and 9 (15%) ended in spontaneous abortion compared to 55 (79%), 14 (20%) and 3 (4%) respectively in the control population. None of the 38 neonates from SLE mothers had neonatal lupus, anti-Ro or anti-La antibodies. Increased titer of ANA was found in one neonate while decreased level of C_3 and C_4 in two of them. B) Influence of pregnancy on SLE: Eight of 40 pregnancies (20%) were characterized by occurrence of a flare of SLE compared to 19 (35%) in the non pregnant SLE group. All flares in the pregnant group responded to prednisone treatment. Arthralgias or arthritis, fever and skin lesions were observed more frequent in the pregnant SLE group compared to the control group ($p<0.001$). Renal involvement was found in two pregnant patients during pregnancy and in four immediately after that.

Conclusion: SLE does not appear to influence the outcome of pregnancy, while pregnancy does not cause SLE to worsen.

1639

CONTRACEPTION IN SYSTEMIC LUPUS ERYTHEMATOSUS. METHODS UTILIZED BY FEMALE PATIENTS. Jorge Sánchez-Guerrero, Juanita Romero-Díaz, Marilú Mestanza-Peralta,and María C. Cravioto-Galindo.Instituto Nacional de la Nutrición Salvador Zubirán. México,D.F.14000 MEXICO.

Aim: Describe contraceptive methods utilized by SLE patients. Methods: Cross sectional survey, face-to-face interview. Subjects: 291 consecutive SLE and 150 RA patients. Statistics: Chi-square, Fisher exact-test Results: From 291 SLE patients (age 36.1±12.1 yrs.), 75 (25.8%) were postmenopausal, 52 (17.9%) have not started sexual life yet, 41 (14.1%) currently do not have sexual activity, and 123 (42.3%) do have. Among 150 RA patients (age 38.3 ± 7.6 yrs.), 24 (16%) were postmenopausal, 17 (11.3%) have not started sexual life yet, 23 (15.3%) currently do not have sexual activity, and 86 (57.3%) do have. Thirteen SLE and 6 RA patients used two methods simultaneously. No significant difference existed between both groups for any of the methods. Hormonal methods were used in 9% of SLE and 4.7% of RA patients.

Methods	SLE	RA
Natural	20 (16.3%)	22 (25.6%)
Local	30 (24.4%)	15 (17.4%)
Intrauterine device	23 (18.7%)	13 (15.1%)
Combined oral	6 (4.9%)	3 (3.5%)
Progestin only pill	4 (3.3%)	0
Injected combined	1 (0.8%)	1 (1.2%)
Injected progestin	0	0
Tubal occlusion	31 (25.2%)	21 (24.4%)
Vasectomy	5 (4.0%)	4 (4.7%)
None	16 (13.0%)	13 (15.1%)

Conclusion: Hormonal contraceptive methods are used infrequently among patients with SLE and RA. A large percentage of SLE and RA patients do not follow an efficient contraceptive method, even when at least in SLE, pregancy is considered of high-risk.

1640

ANTI-NUCLEOSOME ANTIBODIES AS MARKERS FOR RENAL DISEASE IN LUPUS NEPHRITIS. Michele A. Tupchong, Joan Wither, David Hallett, Dafna D. Gladman. Rheumatic Disease Unit, The Toronto Hospital, University of Toronto.

Previous studies have demonstrated the presence of anti-nucleosome antibodies (aNUCL) in patients with SLE, however, their relationship to active lupus nephritis has not been studied. Our objectives were to determine the frequency of elevated levels of anti-nucleosome antibodies in SLE patients and to evaluate the clinical relevance of these antibodies.

128 consecutive lupus patients assessed at the University of Toronto Lupus Clinic between Sept.-Dec. 1996 were enrolled in the study. In addition to the standard clinical-laboratory protocol, blood was drawn for measurement of IgM and IgG aNUCL and anti-dsDNA antibodies by ELISA. Clinical activity was determined by the SLEDAI, a validated measure of disease activity, and active renal disease was defined by the renal descriptors of the SLEDAI.

Elevated levels of aNUCL (IgG, IgM), and anti-dsDNA (IgG, IgM) were detected in: 30/128 (23%), 54/128 (42%), 33/128 (26%) and 29/128 (23%) of patients respectively. There was no correlation between high levels of aNUCL and anti-dsDNA antibodies. The levels of autoantibodies [mean(sd)] for the normal controls and lupus patients were:

	anti-nucleosome		anti-dsDNA	
PATIENTS	IgG	IgM	IgG	IgM
Normal	21.6 (33.3)	27.0 (19.9)	42.8 (37.8)	33.3 (18.7)
SLE	69.4 (79.6)	70.0 (88.5)	99.1 (97.1)	54.8 (49.9)
Active renal	130.9 (158.9)	81.5 (113.5)	208.4 (271.1)	54.4 (34.7)

Elevated levels of aNUCL did not correlate with the SLEDAI. There was a low statistically significant correlation between elevated aNUCL IgG antibody and active lupus nephritis (p=0.054). There was no correlation between anti-dsDNA antibodies and active lupus nephritis (p=0.24).

Thus, there is a trend to increasing frequency of elevated anti-nucleosome antibodies in patients with active lupus nephritis, however, further studies are required before definitive correlations can be made.

1641

AUTOIMMUNE THROMBOCYTOPENIA IN PRIMARY ANTI-PHOSPHOLIPID SYNDROME AND SYSTEMIC LUPUS ERYTHEMATOSUS
Alan J. Hakim, Samuel J. Machin, David A. Isenberg. Depts Medicine/Haematology, University College London, W1P 9PG, U.K.

Thrombocytopenia due to peripheral immune destruction is a recognised complication of systemic lupus erythematosus (SLE) and primary antiphospholipid syndrome (PAPS). Severe immune mediated thrombocytopenia is treated with oral corticosteroids initially; lack of sustained response is followed usually by splenectomy.

An analysis of splenectomy for immune thrombocytopenia associated with SLE or PAPS was performed on adults seen at UCH/Middlesex from 1978 to 1996. Severe immune thrombocytopenia was considered present if platelet counts were below $50x10^9$/L on 2 consecutive occasions with no other cause. Complete remission was defined as platelet counts above $150x10^9$/L for greater than 6 months. Antinuclear antibody, extractable nuclear antigen, rheumatoid factor, anticardiolipin and antiphospholipid antibody (aPL), double stranded DNA antibody and complement titres were documented. Platelet antibodies and bone marrow aspirate were not sought on a regular basis. The British Isles Lupus Assessment Group (BILAG) index was used to record clinical disease activity in SLE patients.

35 patients with PAPS were under review. Of 200 patients with SLE followed for at least 2 years or until death, 33 had thrombocytopenia, 47 had anticardiolipin antibodies and 13 both characteristics. 4 patients with PAPS, 12 with SLE and 1 with 'lupus-like' disease (LLD) had severe thrombocytopenia. 13 required splenectomy: 3 PAPS, 9 SLE (3 with aPL) and 1 LLD. Post splenectomy all patients with PAPS and 6 with SLE gained complete remission with a mean duration of 93 months (range 48-166) in the latter. One with PAPS and 3 with SLE gained partial or complete remission with oral medication and did not require splenectomy. Antibody profile did not change during follow-up; aPL did not influence outcome. There were no complications from splenectomy and no aggravation or induction of more severe disease in either the PAPS or SLE group.

Patients with PAPS or SLE (with or without aPL) can gain long term remission or severe thrombocytopenia after splenectomy. Corticosteroids remain 'first line' treatment, modern surgery, antibiotics and vaccination making splenectomy safer and more viable for patients who do not respond.

PREDNISONE AND ASPIRIN IN WOMEN WITH AUTOANTIBODIES AND UNEXPLAINED RECURRENT FETAL LOSS

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ABSTRACT

Background Recurrent fetal loss has been well described in women with antiphospholipid antibodies. Such women also often have other autoantibodies commonly found in patients with systemic lupus erythematosus. Treating them with prednisone and aspirin may reduce the risk of fetal loss.

Methods We screened 773 nonpregnant women who had the unexplained loss of at least two fetuses for antinuclear, anti-DNA, antilymphocyte, and anticardiolipin antibodies and for the lupus anticoagulant. Of 385 women with at least one autoantibody, 202 who later became pregnant were randomly assigned in equal numbers to receive either prednisone (0.5 to 0.8 mg per kilogram of body weight per day) and aspirin (100 mg per day) or placebo for the duration of the pregnancy. The women were stratified according to age (18 to 34 years or 35 to 39 years) and the week of gestation at which the previous fetal losses had occurred (≤12 or >12 weeks). The primary outcome measure was a successful pregnancy.

Results Live infants were born to 66 women in the treatment group (65 percent) and 57 women in the placebo group (56 percent, $P=0.19$). More infants were born prematurely in the treatment group than in the placebo group (62 percent vs. 12 percent, $P<0.001$). The major side effects of therapy in the mothers were hypertension (treatment group, 13 percent; placebo group, 5 percent; $P=0.05$) and diabetes mellitus (15 percent and 5 percent, $P=0.02$).

Conclusions Treating women who have autoantibodies and recurrent fetal loss with prednisone and aspirin is not effective in promoting live birth, and it increases the risk of prematurity. (N Engl J Med 1997; 337:148-53.)

THE causes of recurrent fetal loss include anatomical, genetic, and hormonal disorders. However, in approximately 60 percent of women the recurrent fetal loss is unexplained. Recurrent fetal loss is a well-known manifestation of several autoimmune diseases. In the case of systemic lupus erythematosus, there is a strong association with fetal loss that has prompted several investigators to propose an autoimmune pathogenesis for otherwise unexplained recurrent fetal loss.[1-5] A nonspecific global inhibitor of in vitro coagulation, the lupus anticoagulant, is associated with fetal wastage in women with systemic lupus erythematosus. This anticoagulant, and numerous other autoantibodies often found in such women, are also found in otherwise normal, healthy women who have recurrent fetal loss.[6-13]

Treatments for these women have included moderate-to-high doses of prednisone and aspirin,[6,7,14-16] on the rationale that the women have a subtle autoimmune disorder that is manifested by recurrent fetal loss. None of these studies have definitely shown this potentially toxic therapy to be either effective or ineffective. We studied the efficacy of prednisone and aspirin in women with autoantibodies and unexplained recurrent fetal loss, with respect to maternal morbidity and fetal survival.

METHODS

We initially evaluated 1080 women from southern Ontario who were referred to our Recurrent Fetal Loss program for this study. We found 773 women who had the unexplained loss of at least two fetuses and thus met the criterion for recurrent fetal loss. Among them, 385 women had at least one repeatedly positive autoantibody test and were deemed eligible for the study when they became pregnant again. Two hundred seventy women agreed to participate in the study. Of these, 202 became pregnant and were randomly assigned to treatment or placebo between February 1988 and November 1994 (Fig. 1). It was established that once pregnant, those eligible for the study would contact the study office immediately.

Selection of Patients

The criteria for inclusion in the study were as follows: an age of 18 to 39 years, at least two consecutive fetal losses before 32 weeks' gestation, and positive results of at least one of the following on at least two of three occasions: activated partial-thromboplastin time test or tests for antinuclear antibodies, anti-DNA antibodies (single- or double-stranded), antilymphocyte IgM, anticardiolipin IgG, or the lupus anticoagulant.

The criteria for exclusion were as follows: a chromosomal or anatomical abnormality or a luteal-phase defect (as determined by

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*Deceased.

a timed endometrial biopsy), which are all known causes of recurrent fetal loss; confirmed peptic ulcer disease within the past three years; systemic lupus erythematosus that fulfilled four or more of the criteria of the American College of Rheumatology[17]; diabetes mellitus, as evidenced by a repeatedly elevated plasma glucose concentration while fasting, abnormal results of a 100-g oral glucose-tolerance test, or previous gestational diabetes mellitus; sensitivity to aspirin; diastolic blood pressure greater than 90 mm Hg on two or more occasions at least three days apart despite antihypertensive therapy; previously untreated tuberculosis, as determined by an abnormal chest film in the previous year or a positive tuberculin skin test; and previous prednisone therapy.

Women deemed eligible for randomization were asked to provide informed consent and were given a package of written information. The study was approved by the Human Experimentation Committee of the University of Toronto. All the women who gave consent had measurements of serum glucose, electrolytes, immunoglobulins, C3, C4, CH50, creatine kinase, aspartate aminotransferase, and rheumatoid factor; Venereal Disease Research Laboratory tests; tests for lupus erythematosus (LE) cells, anti-DNA antibodies, and antithyroid antibodies; a direct Coombs' test; and chest radiography (or a tuberculin skin test). A collaborating ophthalmologist examined the women for cataracts at base line, midway through the pregnancy, and post partum.

Autoantibody Assays

Serum samples were collected on two to three occasions at least 7 to 10 days apart, stored at −20°C, and assayed at the same time in duplicate. If only one of two samples was positive on a given test, a third sample was obtained at least six weeks after the first. Serum levels of anti-DNA single- and double-stranded antibodies (IgG and IgM) and antilymphocyte IgM antibodies were measured with previously described enzyme-linked immunosorbent assays.[18,19] The results were considered positive if the optical-density readings were more than 2 SD above the mean of serum samples from 504 normal, nonpregnant women randomly selected from the general population. An in-house assay[19] and two commercially available kits (Sanofi-Pasteur and INOVA) were used during the eight-year study period to measure anticardiolipin IgG antibodies. Each method was standardized with serum samples of known anticardiolipin IgG (measured in IgG phospholipid [GPL] values) so that the results of the various assays could be compared. When more than 15 to 22 GPL units were measured, the serum samples were considered positive. Antinuclear antibodies were measured with a HEp-2 cell line; a titer of 1:40 or higher was considered positive.

The lupus anticoagulant was considered present if any of the following were prolonged: the partial-thromboplastin time, the Russell's viper-venom time, the kaolin–cephalin clotting time, or the tissue thromboplastin-inhibition time.

Study Protocol

Women who gave consent were instructed to undergo a pregnancy test as soon as their menstrual periods were delayed or a pregnancy was suspected. Positive tests were confirmed by two quantitative measurements of serum levels of the beta subunit of human chorionic gonadotropin at least 24 hours apart that showed an appropriate increase (a doubling of the value every 48 hours) or by ultrasonography that showed a fetus of appropriate size for its gestational age and with a fetal heartbeat. Before randomization, a second, identical consent form was provided, allowing each woman to reconsider her participation in the study.

The randomization was controlled centrally through the study coordinators' office. The medications were packaged in sealed envelopes, and the randomization code was available only to the persons who packaged them and to the biostatistician, none of whom had any contact with the women in the study. Each woman was assigned the next study number in one of four strata, with a balanced four-block procedure to ensure equal numbers in each group as the study progressed. The women were stratified according to age (18 to 34 years or 35 to 39 years) and the week of gestation at which their fetal losses had occurred — early (≤12 weeks) or late (13 to 32 weeks). An interim analysis was performed after 80 women had completed 20 weeks' gestation to determine whether the trial should be terminated because of either side effects or a significant difference ($P \leq 0.01$) between groups with regard to the primary outcome.

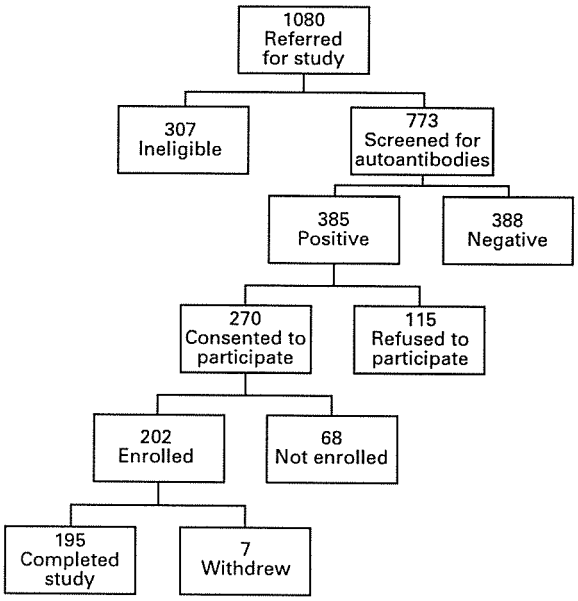

Figure 1. Accrual of Study Subjects with Recurrent Fetal Loss.
Women were evaluated and enrolled in the study between 1988 and 1994. The initial screening included 1080 women, 202 of whom were ultimately enrolled.

All major medical events and the results of the interim analysis were reported to an external safety-monitoring committee composed of consultant specialists.

Drug Administration

The dose of prednisone (Deltasone, Upjohn, Kalamazoo, Mich.) was 0.8 mg per kilogram of body weight per day for four weeks (maximum, 60 mg), followed by 0.5 mg per kilogram per day (maximum, 40 mg) until delivery or fetal loss. At the time of delivery, the attending obstetrician and the woman were informed of the woman's treatment assignment so that glucocorticoid coverage could be begun, all other treatment stopped as appropriate, and the prednisone therapy tapered (decreased by 5 mg every two weeks post partum).

The dose of aspirin (Astrix slow-release, encapsulated aspirin; Faulding Pharmaceuticals, Salisbury, Australia) was 100 mg per day. Aspirin was given until 36 weeks' gestation or shortly before delivery.

Clinical Monitoring

Data on physical symptoms, side effects, and compliance with medication were collected every month throughout the pregnancy. Obstetrical data were collected each trimester.

Laboratory Monitoring

Complete blood counts and tests for all the autoantibodies and the lupus anticoagulant were performed at approximately 8 and 28 weeks' gestation and at delivery. A 50-g glucose-challenge test

TABLE 1. BASE-LINE CHARACTERISTICS OF THE WOMEN IN THE STUDY GROUPS.

CHARACTERISTIC	TREATMENT (N=101)	PLACEBO (N=101)
Mean (±SD) age at randomization — yr	33±3.8	32±4.1
Mean (±SD) no. of previous fetal losses	3.4±1.3	3.5±1.4
	no. of women (%)	
Current smoker	12 (12)	11 (11)
≥1 First-trimester loss	97 (96)	96 (95)
≥1 Loss at 13–20 wk	24 (24)	25 (25)
≥1 Loss at >20 wk	10 (10)	12 (12)
No previous live birth	70 (69)	69 (68)
Previous therapeutic abortion	13 (13)	13 (13)
Previous stillbirth	6 (6)	2 (2)
Previous neonatal death	2 (2)	0
Antinuclear antibodies	37 (37)	46 (46)
Anti-DNA antibodies		
Single-stranded	18 (18)	19 (19)
Double-stranded	13 (13)	19 (19)
Antilymphocyte antibodies	38 (38)	26 (26)
Lupus anticoagulant	38 (38)	36 (36)
Anticardiolipin antibodies	6 (6)	14 (14)

TABLE 2. MAJOR OUTCOMES OF PREGNANCY IN THE STUDY GROUPS, WITH A LOGISTIC-REGRESSION ANALYSIS OF THE EFFECT OF TREATMENT AND OTHER VARIABLES ON OUTCOME.

OUTCOME	TREATMENT (N=101)	PLACEBO (N=101)	P VALUE
	no. (%)		
Live birth	66 (65)	57 (56)	0.19
At term*	25 (38)	50 (88)	<0.001
Before term*	41 (62)	7 (12)	
Fetal loss	35 (35)	44 (44)	

VARIABLE IN REGRESSION ANALYSIS†	PARAMETER ESTIMATE	P VALUE	ODDS RATIO (95% CONFIDENCE INTERVAL)
Treatment vs. placebo	0.38	0.19	1.5 (0.8–2.6)
Older vs. younger maternal age	−0.02	0.94	1.0 (0.5–1.8)
Previous fetal losses (late vs. early)	−0.17	0.58	0.8 (0.5–1.6)

*Percentages shown are based on the total number of live births.

†The stratification of maternal age and the week of gestation at which previous fetal losses occurred is described in the Methods section.

was given at 16, 28, and 32 weeks. If the results of that test were elevated, a 100-g oral glucose-tolerance test was given, and if that result was abnormal (value in the fasting state, >105 mg per deciliter [5.8 mmol per liter]; after one hour, >190 mg per deciliter [10.6 mmol per liter]; after two hours, >165 mg per deciliter [9.2 mmol per liter]; after three hours, >145 mg per deciliter [8.1 mmol per liter]), the woman was referred to an endocrinologist for counseling and treatment.

Evaluation of Efficacy

The primary end point was the survival of the infant for more than one week. The secondary end points included maternal side effects during pregnancy, such as gestational diabetes mellitus, cataracts, epistaxis, hypertension, rash, facial swelling, headaches, hospitalization, and premature birth. For the infants, the end points included the birth weight, Apgar score, and whether there was a need for admission to the neonatal intensive care unit.

Data Collection and Management

At delivery, a form was completed on which information about the labor, delivery, and health of the infant was recorded. Placental disease was evaluated whenever possible. Cranial ultrasonography of the neonates was performed whenever possible, to screen for intraventricular hemorrhage. The laboratory values were measured again six months after fetal loss or delivery.

Statistical Analysis

Two-sample t-tests (two-tailed) were used to test for the equality of the means of continuous variables, and chi-square or Fisher's exact tests were used to test for the equality of proportions in the case of categorical variables. Logistic-regression analysis was used to assess the effect of treatment on the probability of a successful pregnancy, with control for age (35 to 39 years vs. 18 to 34 years) and the week of gestation at which the previous fetal loss had occurred (early or late). The study groups were compared with respect to secondary outcome variables by two-sample t-tests in the case of continuous variables and chi-square or Fisher's exact tests in the case of categorical variables. To assess the safety of treatment with prednisone and aspirin, the number of women who withdrew from the study and the frequency and duration of important adverse events were compared between groups by Fisher's exact test or the chi-square test, depending on the frequency of the events. All the data were entered in a data base (Oracle, Belmont, Calif.) and analyzed with SAS software (SAS Institute, Cary, N.C.).

RESULTS

We enrolled 202 women in the study and subsequently began treating them; there were 101 in each group. Each woman was followed throughout her pregnancy and for at least two years post partum, regardless of the outcome of the pregnancy. Seven women withdrew from the study before delivery (Fig. 1), one because of side effects and the remaining six because they decided not to participate in the study and chose only to continue the follow-up.

The characteristics of the treatment and placebo groups were similar at the time of randomization (Table 1). Forty-four of the 202 women (22 percent) had two fetal losses, and 158 (78 percent) had three or more. Fifty percent of the women originally screened (385 of 773) had at least one autoantibody test that was repeatedly positive (Fig. 1), and 111 of those women (29 percent) had repeatedly positive tests for more than one autoantibody or were posi-

tive for the lupus anticoagulant. The lupus anticoagulant was found in 38 women in the treatment group and 36 in the placebo group; 6 and 14 women, respectively, were positive for anticardiolipin IgG.

Fetal Survival and Other Outcomes of Pregnancy

There were 66 live births (65 percent) in the treatment group and 57 (56 percent) in the placebo group ($P=0.19$) (Table 2). Treatment had no effect after adjustment for maternal age and the week of gestation at which the previous fetal losses occurred (early or late) ($P=0.19$) (Table 2).

Among the women who tested positive for anticardiolipin antibodies or the lupus anticoagulant, 60 percent of those in the treatment group had live births (25 infants were born to 42 mothers), as compared with 52 percent of those in the placebo group (24 infants were born to 46 mothers). Adding an interaction term to the regression analysis (Table 2), in order to relate the presence of anticardiolipin antibody or the lupus anticoagulant to treatment, revealed no significant difference between treatment and placebo in this subgroup ($P=0.81$), indicating that the effect of treatment in the subgroup was not different from that in the group as a whole.

Preterm delivery, premature labor, and premature rupture of the membranes were all significantly more frequent in the treatment group than in the placebo group ($P<0.001$) (Table 2). The women in the treatment group delivered their babies earlier, with most of the births occurring between 32 and 38 weeks of gestation (Fig. 2). All the deliveries after 30 weeks in both groups were of live infants who survived for more than 1 month. All the spontaneous abortions, stillbirths, and neonatal deaths occurred before 23 weeks of gestation. Despite the higher frequency of prematurity in the treatment group, the birth weight of all the neonates in either group was appropriate for their gestational ages — that is, between the 10th and 90th percentiles in almost all cases (Fig. 3). Birth weight below 2500 g was no more frequent in the treatment group than in the placebo group ($P=0.20$). More infants in the treatment group were admitted to the neonatal intensive care unit ($P<0.001$). There was no significant difference between the groups with respect to the incidence of infections or congenital anomalies (Table 3).

Adverse Effects in the Mothers

Hypertension was more common among the women in the treatment group (13 percent, as compared with 5 percent in the placebo group; $P=0.05$), as was gestational diabetes mellitus (15 percent vs. 5 percent, $P=0.02$). One woman in the treatment group withdrew from the study because of gestational diabetes mellitus. Cataracts developed in two

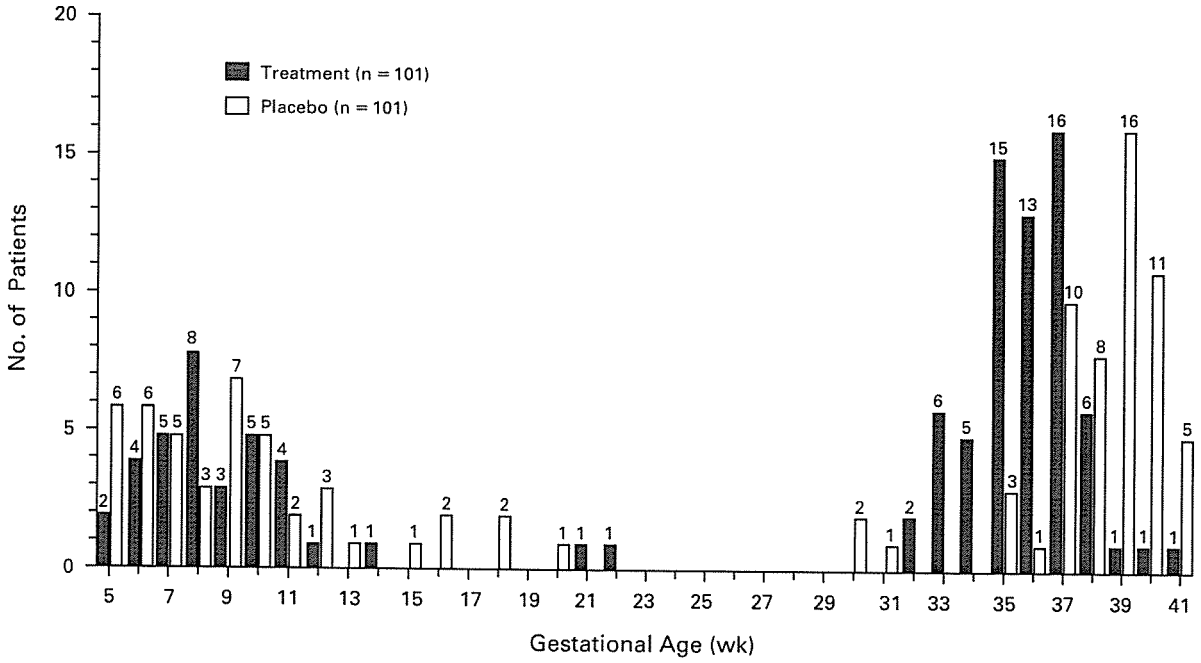

Figure 2. Deliveries in the Treatment and Placebo Groups, According to the Week of Gestation.

Early birth was significantly more common in the treatment group than in the placebo group. All infants born at 30 weeks' gestation or later were born alive. The majority of births in the treatment group occurred between 32 and 38 weeks of gestation, whereas in the placebo group the majority of infants were born between 37 and 41 weeks.

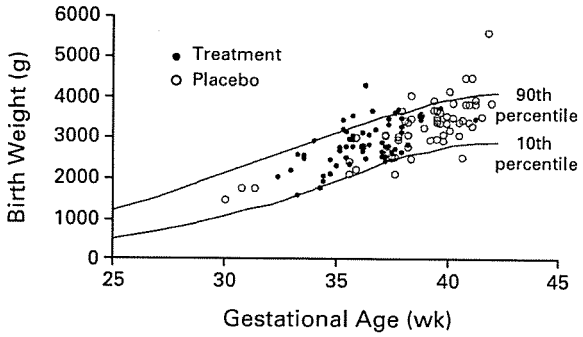

Figure 3. Birth Weights of Infants in the Treatment and Placebo Groups, According to Gestational Age.

Only one infant in the treatment group had a birth weight below the 10th percentile, as compared with three infants in the placebo group. In contrast, eight infants in the treatment group and seven in the placebo group had birth weights above the 90th percentile.

TABLE 3. CHARACTERISTICS AND OUTCOMES OF INFANTS BORN TO MOTHERS IN THE STUDY GROUPS.

VARIABLE*	TREATMENT†	PLACEBO‡	P VALUE
	no./no. studied (%)		
Birth weight <2500 g	17/61 (28)	11/61 (18)	0.20
Male sex	40/64 (62)	28/61 (46)	0.06
Admission to NICU	18/52 (35)	2/54 (4)	<0.001
Days in NICU§	4.4±3.2	6.0±1.4	0.41
Sepsis	1/53 (2)	0/49	1.00
Congenital anomaly	0/56	2/56 (4)	0.50

*NICU denotes neonatal intensive care unit.

†Two pairs of twins are included in this group.

‡Six pairs of twins are included in this group.

§Values shown are means ±SD. Data are based on 18 infants in the treatment group and 2 infants in the placebo group.

women in the treatment group, as compared with none in the placebo group.

DISCUSSION

The presence of circulating autoantibodies and lupus anticoagulant in women with recurrent fetal loss has been well documented.[20-24] Fifty percent of the women screened for this trial had repeatedly positive tests for at least one of the autoantibodies we studied, but none met the American College of Rheumatology criteria for systemic lupus erythematosus[17] or other connective-tissue diseases. We have continued to follow these women by either clinical evaluation or questionnaires. At this writing, rheumatoid arthritis has developed in one and systemic lupus erythematosus in another.

Among the women with recurrent fetal loss who had autoantibodies, prednisone and aspirin were no more effective than placebo in preventing fetal loss during a subsequent pregnancy. Gestational diabetes mellitus and hypertension were important maternal side effects of the therapy, although neither was as frequent as in previous studies.[25,26] In addition to these expected side effects, two women in the treatment group acquired cataracts, which have not progressed on follow-up evaluation. These effects of prednisone and aspirin on the mothers would not have been sufficiently severe to warrant withholding the treatment had it proved effective in preventing fetal loss.

There was a significantly higher incidence of preterm delivery (delivery before 37 weeks' gestation) in the treatment group, in accordance with the findings of previous uncontrolled studies.[27] Although the frequency of prematurity was high, few infants were born before 34 weeks' gestation, and all the neonates treated in the neonatal intensive care unit were discharged without needing readmission. More important, weight was appropriate for gestational age in every infant. Because no increased rate of prematurity was found in two large, randomized trials of low-dose aspirin for the prevention of preeclampsia, it is unlikely that aspirin was responsible for the preterm deliveries.[28,29]

Our study would have been strengthened by the inclusion of a group treated with aspirin alone, but that would have required a considerably larger study. Also, if aspirin alone were an effective treatment, the prednisone would have had to have a blunting effect, a possibility we consider unlikely.

Our determination of the effect of treatment on women with high levels of anticardiolipin antibodies, late fetal loss, and other manifestations of the antiphospholipid-antibody syndrome was limited by the size of our sample. Interaction analysis did not show a greater response to treatment in this subgroup. Few women with these characteristics were identified among the 1080 women initially screened for this study.

We conclude that prednisone and aspirin are not effective in preventing fetal loss in women with serum autoantibodies and a history of recurrent fetal loss.

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REFERENCES

1. Friedman EA, Rutherford JW. Pregnancy and lupus erythematosus. Obstet Gynecol 1956;8:601-10.
2. Fraga A, Mintz G, Orozco J, Orozco H. Sterility and fertility rates, fetal wastage and maternal morbidity in systemic lupus erythematosus. J Rheumatol 1974;1:293-8.
3. Cecere FA, Persellin RH. The interaction of pregnancy and the rheumatic diseases. Clin Rheum Dis 1981;7:747-68.
4. Merrill JA. Cortisone in disseminated lupus erythematosus during pregnancy. Obstet Gynecol 1955;6:637-43.
5. Ramsey Goldman R. Pregnancy in systemic lupus erythematosus. Rheum Dis Clin North Am 1988;14:169-85.
6. Lubbe WF, Butler WS, Palmer SJ, Liggins GC. Fetal survival after prednisone suppression of maternal lupus-anticoagulant. Lancet 1983;1:1361-3.
7. Branch DW, Kochenour NK, Hershgold EJ, Rote NS, Scott JR. The lupus anticoagulant — a recently discovered and treatable cause of recurrent abortion and fetal death. Am J Reprod Immunol 1984;5:100. abstract.
8. Unander AM, Norberg R, Hahn L, Arfors L. Anticardiolipin antibodies and complement in ninety-nine women with habitual abortion. Am J Obstet Gynecol 1987;156:114-9.
9. Cronin ME, Biswas RM, Van der Straeton C, Fleisher TA, Klippel JH. IgG and IgM anticardiolipin antibodies in patients with lupus with anticardiolipin antibody associated clinical syndromes. J Rheumatol 1988;15: 795-8.
10. Harris EN, Chan JK, Asherson RA, Aber VR, Gharavi AE, Hughes GRV. Thrombosis, recurrent fetal loss, and thrombocytopenia: predictive value of the anticardiolipin antibody test. Arch Intern Med 1986;146: 2153-6.
11. Lockshin MD, Druzin ML, Goei S, et al. Antibody to cardiolipin as a predictor of fetal distress or death in pregnant patients with systemic lupus erythematosus. N Engl J Med 1985;313:152-6.
12. Cowchock S, Smith JB, Gocial B. Antibodies to phospholipids and nuclear antigens in patients with repeated abortions. Am J Obstet Gynecol 1986;155:1002-10.
13. Parke A, Maier D, Hakim C, Randolph J, Andreoli J. Subclinical autoimmune disease and recurrent spontaneous abortion. J Rheumatol 1986; 13:1178-80.
14. Forouzan I, Cohen AW, Lindenbaum C, Samuels P. Umbilical artery and ductal blood flow velocities in patients treated with aspirin and prednisone for presence of anticardiolipin antibody. J Ultrasound Med 1993; 12:135-8.
15. Lockshin MD, Druzin ML, Qamar T. Prednisone does not prevent recurrent fetal death in women with antiphospholipid antibody. Am J Obstet Gynecol 1989;160:439-43.
16. Passaleva A, Massai G, D'Elios MM, Livi C, Abbate R. Prevention of miscarriage in antiphospholipid syndrome. Autoimmunity 1992;14:121-5.
17. Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25: 1271-7.
18. Laskin CA, Vidins E, Blendis LM, Soloninka CA. Autoantibodies in alcoholic liver disease. Am J Med 1990;89:129-33.
19. Soloninka CA, Laskin CA, Wither J, Wong D, Bombardier C, Raboud J. Clinical utility and specificity of anticardiolipin antibodies. J Rheumatol 1991;18:1849-55.
20. Gleicher N. Pregnancy and autoimmunity. Acta Haematol 1986;76: 68-77.
21. Hawker GA, Bombardier C, Soloninka CA, et al. Autoantibodies in healthy women and in women with a history of unexplained recurrent fetal loss. Arthritis Rheum 1991;34:S95.
22. Gleicher N, el-Roeiy A, Confino E, Friberg J. Reproductive failure because of autoantibodies: unexplained infertility and pregnancy wastage. Am J Obstet Gynecol 1989;160:1376-80.
23. Love PE, Santoro SA. Antiphospholipid antibodies: anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders. Ann Intern Med 1990;112:682-98.
24. Rai RS, Regan L, Clifford K, et al. Antiphospholipid antibodies and β2-glycoprotein-I in 500 women with recurrent miscarriage: results of a comprehensive screening approach. Hum Reprod 1995;10:2001-5.
25. Cowchock FS, Reece EA, Balaban D, Branch DW, Plouffe L. Repeated fetal losses associated with antiphospholipid antibodies: a collaborative randomized trial comparing prednisone with low-dose heparin treatment. Am J Obstet Gynecol 1992;166:1318-23.
26. Branch DW, Silver RM, Blackwell JL, Reading JC, Scott JR. Outcome of treated pregnancies in women with antiphospholipid syndrome: an update of the Utah experience. Obstet Gynecol 1992;80:614-20.
27. Silver RK, MacGregor SN, Sholl JS, Hobart JM, Neerhof MG, Ragin A. Comparative trial of prednisone plus aspirin versus aspirin alone in the treatment of anticardiolipin antibody-positive obstetric patients. Am J Obstet Gynecol 1993;169:1411-7.
28. Sibai BM, Caritis SN, Thom E, et al. Prevention of preeclampsia with low-dose aspirin in healthy, nulliparous pregnant women. N Engl J Med 1993;329:1213-8.
29. CLASP: a randomised trial of low-dose aspirin for the prevention and treatment of pre-eclampsia among 9364 pregnant women. Lancet 1994; 343:619-29.

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ISOTYPES OF ANTI-β_2-GLYCOPROTEIN I ANTIBODIES: ASSOCIATION WITH THROMBOSIS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS.
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Detection of anti-cardiolipin antibody(aCL) has been known to be useful for predicting thrombotic events in patients with SLE. There are few reports on the significance of isotypes of anti-β_2-glycoprotein I antibodies($a\beta_2$GPI). To evaluate the clinical significance and to search for a relationship between the presence of IgG aCL and $a\beta_2$GPI, IgG aCL and isotypes of $a\beta_2$GPI were measured by ELISA and clinical evidence of thrombosis was examined in 267 patients with SLE.

IgG aCL and lupus anticoagulant(LAC) were positive in 28.6% and 17.8% respectively. The arterial thrombosis was associated with presence of IgG aCL, whereas venous thrombosis was associated with LAC. IgG, IgM, and IgA $a\beta_2$GPI were positive in 20.5%, 13.7%, and 18.6%, respectively. Each isotype of $a\beta_2$GPI was variably associated with an increased frequency of both arterial and venous thrombosis. In multivariate analyses, significant predictor of arterial thrombosis was IgG $a\beta_2$GPI and that of venous thrombosis was IgA $a\beta_2$GPI. The titers of IgG, IgM, and IgA $a\beta_2$GPI were closely correlated with those of IgG aCL(r=0.653, p<0.001, r=0.434, p<0.001, r=0.547, p<0.001). The titers of IgA $a\beta_2$GPI were also significantly correlated with those of IgG and IgM $a\beta_2$GPI(r=0.587, p<0.001, r=0.460, p<0.001).

These results suggest that isotypes of $a\beta_2$GPI are related to the occurrence of thrombosis and measurements of $a\beta_2$GPI may be useful for predicting thrombotic episodes in patients with SLE.

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BETA-2-GLYCOPROTEIN I INDUCES LEAKAGE FROM CARDIOLIOPIN-CONTAINING VESICLES BY A NON-FUSION MECHANISM: BINDING, BILAYER UNSTEADY, AGGREGATION AND LEAKAGE.
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Beta-2-glycoprotein I (β_2GPI), a normal plasmatic apolipoprotein, binds to negatively charged phospholipids (PL) such as cardiolipin (CL). We have previously demonstrated that β_2GPI induces the internal content leakage of CL-containing vesicles and induces a sinergistic increase of the leakage rate induced by antiphospholipid antibodies (aPL) from systemic lupus erythematosus (Lupus 5 (1996) 544). A 120 min-delay for the leakage onset was independent of β_2GPI concentration. The objective of this study was elucidate the mechanism by which β_2GPI induces the vesicle leakage. Methods: Phosphatidylcholine (PC)/CL 1:1 (molar) unilamellar vesicles were prepared by sonication containing: (1) entrapped carboxyfluorescein (CF) for leakage trial; (2) pyrene-labeled PC for fusion assays, or (3) spin labeled probes for β_2GPI-bilayer interaction. Human β_2GPI was purified by acid treatment and heparin-Sepharose column. Results: At a constant [β_2GPI]/[vesicle] ratio, CF-leakage rate was comparable to 3-fold higher (or lower) β_2GPI concentrations, indicating an intervesicular binding of β_2GPI. Although β_2GPI aggregates vesicles, no phospholipid mixing was detected, indicating a non-fusion mechanism. Likewise Ca^{2+} effects, β_2GPI decreased the PL mobility near the head group region (using 5-C-spin label PC) and increased PL fluidity in the bilayer core (using 12-C spin label PC). Conclusions: β_2GPI binding to mixed bilayers induces local PL rearrangement leading to PL phase separation. Consequently, dehydration and membrane unsteadies induce vesicle aggregation. The onset delay in the leakage kinetic is explained by the time required for vesicle aggregation induced by β_2GPI and measurable amounts of released probe. The domain formation (phase separation) explains the cofactor activity of β_2GPI for autoimmune aPL.

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ANTI-B2 GLYCOPROTEIN I AND ANTI-CARDIOLIPIN ANTIBODIES DISTINGUISH CLINICAL SUBSETS WITHIN THE ANTI-PHOSPHOLIPID ANTIBODY SYNDROME.
CA Clark-Soloninka, IN Bruce, KA Spitzer, J Nadler, CA Laskin. University of Toronto, Toronto, Ontario, Canada.

Purpose: To determine the relationship of laboratory markers to clinical manifestations in primary anti-phospholipid antibody syndrome (APS).

Study Design: Eighty-four consecutive patients presenting with unexplained recurrent pregnancy loss (RPL) were evaluated for anti-cardiolipin IgG (aCL), the lupus anticoagulant (LAC) and B2 glycoprotein 1 IgG and M (B2 GP1). Thirteen patients with previously documented APS with a history of thrombosis with or without RPL, were also tested. LAC were measured using a panel including dilute PT, kaolin-cephalin clotting time, Russell's Viper venom time and the PTT-LA.

Results Thirty of the 84 women with RPL were positive for LAC and/or aCL (ie. primary APS according to the proposed classification criteria). Both aCL and B2 GP1 were far more frequently found in the APS patients with thrombosis than in the APS group with RPL as the only clinical manifestation.

Group (n)	aCL IgG (%)	B2 GP1 (%)	LAC (%)
RPL (84)	3 (4)	4 (5)	30 (36)
APS (30) (RPL Only)	3(10)	4(13)	30 (100)
APS (13) (Thrombosis + RPL)	6 (46)	9 (70)	7 (54)

Conclusions B2 GP1 was present in 13% of RPL/APS patients compared to 70% of APS patients with thrombosis with or without RPL. A similar lack of association with aCL was found. In the context of primary APS therefore, B2 GP1 and cardiolipin antibodies appear to be markers of hypercoagulability rather than pregnancy loss.

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THE LUPUS ANTICOAGULANT IS FREQUENTLY UNDETECTED BY ROUTINE PTT ASSAYS
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Purpose: To determine the sensitivity of the routine aPTT in the detection of the lupus anticoagulant (LAC). The LAC is frequently found in patients with systemic lupus erythematosus (SLE) and the antiphospholipid antibody syndrome (APS). The LAC has also been reported in some women who experience unexplained recurrent pregnancy loss (RPL), and may be a factor underlying the fetal demise.

Study Design: 590 consecutive women presenting with unexplained RPL, SLE or APS were tested for the LAC using both the anti-cardiolipin IgG (aCL) ELISA and a panel of coagulation tests including the kaolin-cephalin clotting time, the Russell's Viper venom time, the dilute-PT and the PTT-lupus anticoagulant (PTT-LA). Plasma samples from all women were also sent to routine diagnostic labs for PTT testing.

Results: 117/590 (19.8%) had a positive LAC using the panel of assays including 84/590 that had a prolonged PTT-LA. In comparison, only 21/590 (3.6%) had a prolonged PTT result reported from a routine diagnostic lab. Routine tests therefore missed 95/117 (81.2%) of LAC positive plasma samples. All specimens with a prolonged PTT (21/21) were also positive for the PTT-LA.

Conclusions: The LA-sensitive PTT was the most frequently positive test in our LAC panel. The proper evaluation of the LAC must involve a panel of coagulation assays, or at a minimum, the PTT-LA that is specifically designed to detect the LAC and controls for the presence of other factors that might prolong the test. The routine PTT was an inadequate measure of the LAC as it failed to detect more than 80% of LAC positive samples.

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EVALUATION OF SCREENING 590 PLASMA SAMPLES FOR THE LUPUS ANTICOAGULANT USING A PANEL OF FOUR TESTS.
CA Clark-Soloninka, KA Spitzer, J Nadler, CA Laskin. University of Toronto, Toronto, Ontario, Canada.

Purpose: To evaluate a panel of 4 assays for the lupus anticoagulant (LAC) and determine the diagnostic value of each as well as possible correlation with clinical manifestations.

Study Design: 590 plasma samples from women with systemic lupus erythematosus, anti-phospholipid antibody syndrome and unexplained recurrent pregnancy loss were tested for the LAC. The LAC panel included the dilute PT, Russell's Viper venom time (DRVVT), the lupus-specific PTT (PTT-LA), and the kaolin-cephalin clotting time (KCT). Correlations with spontaneous abortions (SA), a history of thrombosis and prolonged routine PTT were observed.

Results: 117 women had at least one positive LAC test. The PTT-LA was the most frequently observed (84/117) followed by the DRVVT (50/117), the KCT (41/117) and the dilute PT (37/117).

Assay (n pos)	SA ≥ 2 (%)	Hx/o Thrombosis (%)	Routine aPTT (%)
Dilute PT (37)	16 (43)	9 (24)	15 (41)
DRVVT (50)	33 (66)	12 (24)	18 (36)
PTT-LA (84)	54 (64)	13 (15)	17 (20)
KCT (41)	23 (56)	8 (20)	14 (34)

Conclusions: In the LAC positive group, the 50/78 women with RPL were positive for only one of the panel, whereas women with a history of thrombosis were more likely to be positive for 2 or more of the panel (12/14 or 86%). Although the PTT-LA was the most frequently positive test, it was not correlated with any particular clinical manifestation. Therefore, all 4 tests are necessary to determine the presence of the LAC, regardless of the clinical presentation.

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ANTICARDIOLIPIN AND ANTI-β2-GLYCOPROTEIN I ANTIBODIES AND ASSOCIATION WITH MYOCARDIAL INFARCTION. A PROSPECTIVE STUDY OF 1153 PATIENTS.
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Objectives: To investigate the prevalence of anticardiolipin antibodies (aCLA) and anti-β2-glycoprotein I (aβ2GPI) antibodies in a cohort of patients with acute myocardial infarction (MI) and their association with recurrent coronary events.

Methods: Sera were drawn at entry from patients with acute MI participating in the THROMBO study (a multicenter, prospective study of 1162 patients with acute MI and 2-year follow-up for recurrent coronary events). Sera were available from the pre-discharge period and at 2-months after the MI. Initial sera were tested for IgG and IgM isotype aβ2GPI antibodies by ELISA (INOVA Diagnostics, positive value >20 standard units (SU)) and are currently being tested for IgG and IgM aCLA. The end-points of the study are cardiac death or non-fatal myocardial infarction. The risk is to be estimated by Cox's regression model.

Results: 1153 sera from the pre-discharge period were available. There were a total of 47 sera with detectable IgG (9 >20 SGU) and 427 sera with detectable IgM (46>20 SMU) aβ2GPI antibodies. Of the 9 IgG and 47 IgM positive pre-discharge sera, 6 and 37 sera respectively were available for analysis at 2-months after the MI. There was no significant change in the antibody concentration between the pre-discharge and the 2-month specimens for either the IgG (p=0.47) or the IgM (p=0.13) isotype. No antibody switch from IgM to IgG isotype was detected from the pre-discharge to the 2-month samples.

Conclusions: aβ2GPI antibodies were positive in 0.7% (IgG) and 3.9% (IgM) of post-MI patients with a stable pattern in the 2-month post-MI period. By design the final data will be analyzed in the context of the THROMBO study and the association of elevated aβ2GPI antibodies and aCLA with recurrent coronary events will be available for presentation at the time of the ACR meeting.

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Prevalence of Antibodies to $ß_2$-Glycoprotein I in Systemic Lupus Erythematosus and Their Association with Antiphospholipid Antibody Syndrome Criteria: A Single Center Study and Literature Review

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ABSTRACT. *Objective.* To determine the prevalence of anti-$ß_2$-glycoprotein I antibodies (anti-$ß_2$-GPI) in patients with systemic lupus erythematosus (SLE), and to assess their association with and predictive value for the clinical classification criteria of the antiphospholipid antibody syndrome (APS).

Methods. One hundred thirty-three consecutive patients with SLE were recruited from 2 lupus clinics in the University of Toronto. Serum and plasma samples were tested for IgG anticardiolipin antibodies (aCL), prolonged partial thromboplastin time (PTT), a panel of lupus anticoagulant (LAC) assays, and anti-$ß_2$-GPI (IgG, IgM, IgA). Normal ranges for the assays were established using 129 healthy controls. A literature review from 1992 to 2000 was performed using $ß_2$-GPI, SLE, APS, thrombosis, and recurrent pregnancy loss as key search words.

Results. The distribution of anti-$ß_2$-GPI antibodies (of any isotype) in each group were as follows: all patients with SLE, 36.8%; SLE with clinical features of APS, 40.4%; SLE without clinical features of APS, 34.9%; and healthy controls, 3%. The positive predictive values of prolonged PTT, IgG aCL, and anti-$ß_2$-GPI for at least one clinical feature of APS in SLE were 59.3, 50.0, and 38.8%, respectively. There were 27 patients with SLE who had antibodies to $ß_2$-GPI but a normal PTT and negative aCL and LAC. Six (20.7%) of these had a history of thrombosis and/or recurrent pregnancy loss. Twelve studies (including ours) were identified in which patient groups were similar and the same antibody isotype was measured. No agreement was apparent after reviewing the literature regarding an association of anti-$ß_2$-GPI IgG and clinical features of APS in patients with SLE.

Conclusion. Antibodies to $ß_2$-GPI were frequently seen (35%) in our SLE population. The prevalence of anti-$ß_2$-GPI was similar in those with (19/47) and without (39/86) APS. Anti-$ß_2$-GPI did, however, identify 6 patients with clinical features of APS who were negative for aCL and prolonged PTT. Our results indicate that anti-$ß_2$-GPI may provide additional information for the diagnosis of APS in SLE, but do not supercede other established assays. However, when we attempted to place our results in the context of other reports, the literature review revealed that secondary diagnoses of patient groups and assay techniques are too variable among different investigators to allow useful comparison. Thus, no conclusions could be drawn regarding anti-$ß_2$-GPI and clinical features of secondary APS in SLE. (J Rheumatol 2000;27:2833–7)

Key Indexing Terms:
ANTI-$ß_2$-GLYCOPROTEIN I
ANTIPHOSPHOLIPID SYNDROME
SYSTEMIC LUPUS ERYTHEMATOSUS
RECURRENT PREGNANCY LOSS
THROMBOSIS

Systemic lupus erythematosus (SLE) is a multifactorial disease of unknown etiology typically involving the expression of myriad autoantibodies. There is also a high coincidence of antiphospholipid syndrome (APS). Proposed classification criteria for APS require the presence of both a clinical manifestation [thromboembolism and/or recurrent pregnancy loss (RPL)] and a positive laboratory test [lupus anticoagulant activity (LAC)], and/or antibodies to cardiolipin (aCL)[1,2].

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APS can occur in isolation (primary APS) or in combination with other autoimmune conditions (secondary APS), especially SLE.

A study has shown that, in SLE, aCL are most strongly associated with prolonged partial thromboplastin time (PTT), thrombocytopenia, and a positive Coomb's test[3]. In that study, a prolonged PTT but not aCL was associated with venous and arterial thrombosis. A subsequent metaanalysis confirmed that, in SLE, LAC was more strongly associated with risk of venous thrombosis than aCL, although both were statistically significant[4].

Beta 2 glycoprotein I ($ß_2$-GPI) is a naturally occurring procoagulant protein that appears to be the cofactor in aCL assays necessary for the binding of certain aCL, although its exact physiological role is still unclear[5]. Antibodies to $ß_2$-GPI (anti-$ß_2$-GPI) have also been identified in the serum of some patients with SLE and/or APS, but as yet no definitive associations with any particular clinical manifestation or laboratory variable have been revealed despite numerous investigations.

We investigated the prevalence of anti-$ß_2$-GPI in a cohort of patients with SLE and compared the frequency of anti-$ß_2$-GPI with aCL and LAC in patients with and without the clinical manifestations of secondary APS. We also compared our results to previous reports and attempted to establish a consensus regarding the significance of anti-$ß_2$-GPI in patients with thromboses and RPL in the context of SLE.

MATERIALS AND METHODS

Patients. One hundred thirty-three consecutive patients from 2 clinics associated with the University of Toronto were studied between March and June 1997. All patients met the 1982 American College of Rheumatology (ACR) criteria for the classification of SLE[6]. At the time of study, all patients underwent a complete history and examination. In addition, their charts were reviewed for previous clinical and serological features of APS using proposed criteria[1,7,8] that included thromboembolism and/or RPL in combination with a positive LAC or aCL test on 2 separate occasions at least 8 weeks apart. Venous thrombosis was documented by ultrasound or venography and arterial thrombosis was confirmed by angiography, or in the case of stroke by computerized tomography (CT) or magnetic resonance imaging (MRI) showing infarction. Myocardial infarction was confirmed by a history of typical chest pain associated with electrocardiographic changes and elevated creatinine kinase. RPL was defined as ≥ 2 consecutive fetal losses in the absence of documented hormonal, anatomic, or genetic abnormalities.

aCL and anti-$ß_2$-GPI assays. Antibodies were measured in duplicate using Inova Quantalite kits (Intermedico, Mississauga, ON, Canada) for the determination of aCL IgG and anti-$ß_2$-GPI IgG, IgM, and IgA. Upper limits of normal were established using 129 sera from healthy controls.

LAC. The LAC was determined by the presence of a either a prolonged PTT or a prolonged result in a panel of coagulation tests including the PTT-LAC, Russell's viper venom time, the kaolin clotting time, and a dilute prothrombin time. Prolonged results were confirmed by repeat testing with 1:1 and 4:1 dilutions with normal plasma.

Statistical analysis. Distributions within groups were described by means and standard deviations (SD). Sensitivity, specificity, and positive predictive values (PPV) were also calculated for each antibody and each clinical feature.

Literature review. A literature search from 1992 to 2000 was performed using $ß_2$-GPI, SLE, APS, recurrent pregnancy loss, and thrombosis as key words. Those studies that included patients with SLE fulfilling the ACR criteria[5] measured aCL using standardized control sera and also measured the prevalence of anti-$ß_2$-GPI IgG were considered appropriate for comparison. In addition, we looked for studies that reported the incidence of both antibodies in patients with and without clinical features of secondary APS. We carefully evaluated the means by which patient classifications were determined, paying particular attention to the clinical and laboratory measures used by the different investigators, to ensure consistency.

RESULTS

Upper limits of normal for each of the isotypes of anti-$ß_2$-GPI were determined to be 15, 20, and 15 units for IgG, IgM, and IgA, respectively, based upon 2 standard deviations above the mean values determined using 129 normal sera tested in triplicate. These values corresponded to those recommended by the manufacturer.

Clinical and laboratory features of 133 patients with SLE are displayed in Table 1. The LAC and aCL were present in 20.3 and 13.5% of patients, respectively.

Forty-nine (36.8%) patients with SLE had anti-$ß_2$-GPI of at least one isotype. There was no difference between those who were positive or negative for anti-$ß_2$-GPI with regard to demographic features or disease activity (Table 2). However, aCL and LAC were more frequently seen in the anti-$ß_2$-GPI positive group. Venous and/or arterial thromboses were not asso-

Table 1. Demographic data of patients with SLE.

Variable	SLE (n = 133)
Sex F/M	124/9
Mean age (range)	39 (18–81)
Mean years disease duration (range)	10 (0–44)
Mean SLEDAI (range)	4 (0–20)
History of	
Venous thrombosis (%)	23 (17.3)
Arterial thrombosis (%)	16 (12.0)
RPL (%)	13 (9.8)
Positive LAC (%)	27 (20.3)
Positive aCL IgG (%)	18 (13.5)

SLEDAI: Systemic Lupus Erythematosus Disease Activity Index, RPL: recurrent pregnancy loss.

Table 2. Comparison of demographic, clinical, and laboratory features of patients with SLE with or without anti-$ß_2$-GPI.

Variable	SLE with $ß_2$-GPI, n = 49	SLE without $ß_2$-GPI, n = 87
Sex F/M	47/2	77/7
Mean age, yrs (range)	37 (19–72)	39 (18–81)
Mean disease duration, yrs (range)	11 (0–27)	11 (1–44)
Mean SLEDAI (range)	4 (0–16)	4 (0–20)
History of		
Venous thrombosis (%)	10 (20.4)	13 (15.5)
Arterial thrombosis (%)	5 (10.2)	11 (13.1)
RPL (%)	8 (16.3)	5 (6.0)
LAC (%)	18 (36.7)	9 (10.7)
aCL IgG (%)	14 (28.6)	4 (4.8)

ciated with the presence of anti-$ß_2$-GPI. A trend toward RPL was seen more often in the antibody positive group, but the sample sizes were so small [only 13 of 133 patients with SLE had a history of RPL, with 8 (16.3%) in the anti-$ß_2$-GPI positive group and 5 (6%) in the anti-$ß_2$-GPI negative group] that the observation has limited value.

Table 3 compares laboratory variables in patients with and without the clinical manifestations of secondary APS. Half of the group with no clinical features had no laboratory finding, but the same was true for the group with at least one clinical finding. The highest incidence of anti-$ß_2$-GPI was in the RPL group (8/13). Eight patients were positive for anti-$ß_2$-GPI, aCL, and LAC, of whom 7/8 (87.5%) had a history of either thrombosis or RPL or both. In contrast, only 1/89 (1.1%) of patients with no clinical features were positive for all 3 antibodies.

The prevalence of each isotype of anti-$ß_2$-GPI antibody was 15.8, 23.3, and 10.5% for IgG, IgM, and IgA, respectively (Table 4). IgG anti-$ß_2$-GPI was the most frequently seen isotype in association with RPL (4/21). Of the 49 patients who were positive for anti-$ß_2$-GPI, 17 (34.7%) had more than one isotype. Of the other 32 patients positive for only one isotype of anti-$ß_2$-GPI, the isotype distribution was as follows: 7 (14.3%) IgG only, 20 (40.8%) IgM only, and 7 (14.3%) IgA only. There was no increased association of clinical features when only high titer antibodies were considered (data not shown).

We also assessed the sensitivity, specificity, and PPV for each antibody with each individual clinical feature of APS in SLE and with the presence of at least one of the features (Table 5). The most sensitive of the 3 tests for detecting a history of RPL was anti-$ß_2$-GPI (61.5%), but it did not reach a useful level. The LAC and aCL both had high specificity for venous and arterial thrombosis and RPL (over 81% each). The PPV were all < 33.3% for each antibody, comparing individual clinical features. However, the PPV for each antibody in combination with at least one clinical feature were higher. The LAC had the highest PPV for the presence of at least one clinical feature of secondary APS (59.3%).

Table 5. Sensitivities, specificities, and positive predictive values for aCL, LAC, and anti-$ß_2$-GPI and clinical features of secondary APS in SLE.

Antibody Clinical Feature	Sensitivity	Specificity	Positive Predictive Value
$ß_2$-GPI			
Venous thrombosis	43.5	64.5	20.4
Arterial thrombosis	31.3	62.4	10.2
RPL	61.5	65.8	16.3
≥ 1 feature	43.2	66.3	38.8
LAC			
Venous thrombosis	39.1	83.6	33.3
Arterial thrombosis	31.3	81.2	18.5
RPL	53.8	83.3	25.9
≥ 1 feature	36.4	87.6	59.3
aCL			
Venous thrombosis	17.4	87.8	22.2
Arterial thrombosis	18.8	87.0	16.7
RPL	30.8	87.8	22.2
≥ 1 feature	20.5	89.9	50.0

Table 3. Coincidence of laboratory variables and clinical features of APS in patients with SLE.

Laboratory Variable	No Clinical Features, n = 89 (%)	Venous Thrombosis, n = 23 (%)	Arterial Thrombosis, n = 16 (%)	RPL, n = 13 (%)	≥ 1 Clinical Feature, n = 44 (%)
No positive antibodies	51 (57.3)	10 (43.5)	9 (56.3)	4 (30.8)	21 (47.7)
$ß_2$-GPI positive	30 (33.7)	9 (39.1)	6 (37.5)	8 (61.5)	19 (43.2)
aCL IgG positive	9 (10.1)	4 (17.4)	3 (18.8)	4 (30.8)	9 (20.5)
LAC positive	11 (12.4)	9 (39.1)	5 (31.3)	7 (53.8)	16 (36.4)
All 3 positive	1 (1.1)	2 (8.7)	3 (18.8)	3 (23.1)	7 (15.9)
≥ 1 positive	38 (42.7)	13 (56.5)	7 (43.8)	9 (69.2)	23 (52.3)

Table 4. Association of anti-$ß_2$-GPI isotypes with clinical features of APS in patients with SLE.

Clinical Feature	$ß_2$-GPI IgG, n = 21 (%)	$ß_2$-GPI IgM, n = 31 (%).	$ß_2$-GPI IgA, n = 14 (%)
Venous thrombosis	4 (19.0)	8 (25.8)	2 (13.3)
Arterial thrombosis	4 (19.0)	4 (12.9)	1 (6.7)
RPL	4 (19.0)	1 (6.7)	1 (6.7)
No clinical feature	12 (57.1)	18 (58.1)	11 (78.6)

DISCUSSION

We found that between 35 and 40% of our patients with SLE have anti-$ß_2$-GPI of at least one isotype regardless of the presence of clinical features of APS. This suggests that in SLE measurement of anti-$ß_2$-GPI does not supercede aCL or LAC, which have both been frequently associated with this syndrome.

Anti-$ß_2$-GPI may, however, provide additional diagnostic information. Six of 27 (20.7%) patients with SLE who were positive for anti-$ß_2$-GPI and negative for LAC and IgG aCL had a history of thrombosis or RPL. This confirms the observation[1,9-11] that there is a group of patients with SLE who manifest clinical symptoms of APS but have not previously had a measurable circulating antiphospholipid antibody. The use of the $ß_2$-GPI assay, therefore, might provide an increased catchment population for identifying secondary APS rather than relying solely on either aCL or LAC positivity.

However, as the prevalence was the same in our patients both with and without clinical manifestations of APS, our data seem to indicate that anti-$ß_2$-GPI antibodies alone are probably unrelated to the clinical manifestations of APS (venous and arterial thromboses and/or a history of RPL) in patients with SLE. Although they are more often than not found in combination with other APS associated laboratory measures (aCL and the LAC), the presence of anti-$ß_2$-GPI is not dependent upon them. We did find that the presence of all 3 antibodies (LAC, aCL, and anti-$ß_2$-GPI), although uncommon (only 8/133 patients, Table 3), was strongly associated with a history of thrombosis and/or RPL.

To determine if our results regarding the clinical associations with anti-$ß_2$-GPI reflect current understanding, we performed a comprehensive literature review. There are at least 20 reports of anti-$ß_2$-GPI and SLE and/or APS. It is difficult to draw conclusions from the literature because patient selection criteria and assay techniques vary from study to study. For example, 3 reports[12-14] all describe the prevalence of anti-$ß_2$-GPI in patients with SLE. However, Day, *et al*[12] tested for antibodies directed against a proprietary mixture of phospholipids instead of measuring aCL, which is the accepted test for this population and which has been standardized with generally distributed reference sera. Day's results therefore are not directly comparable and were not included. Fanopoulos, *et al*[13] preselected their patients with SLE for positive anti-DNA or anti-Sm antibodies, and Sanfilippo, *et* al[14] selected patients with SLE who were all aCL positive. They do not therefore comprise the same heterogeneous groups of patients with SLE investigated by us and others.

Our results and those from 11 other studies[9-11,15-22] that included patients with SLE and measurement of anti-$ß_2$-GPI IgG and aCL IgG are shown in Table 6. The sample sizes range from 20 to 308 with a median of 102. The median percentage positive for aCL IgG and anti-$ß_2$-GPI IgG are 41.9 (range 12.3–70.2%) and 17.1% (range 5.0–40.7%), respectively. If we assume that all patients fulfilled the ACR classification criteria for SLE and that all assays were appropriately performed with adequate controls, then we can combine simple prevalence results. Thus a total of 1342 patients with SLE were tested for both aCL and anti-$ß_2$-GPI IgG. Of that total, 436 (32.5%) are positive for aCL IgG and 246 (18.3%) are positive for anti-$ß_2$-GPI IgG.

Five studies including ours do not report the presence of secondary APS[9,17,21,22]. Of the 7 that do, 4 of them[16,18-20] found an association between the presence of anti-$ß_2$-GPI and secondary APS; 3 studies[10,11,15] do not.

Six of 12 studies (including ours)[10,11,15,18,22] found no association and 6 of 12[9,16,17,19-21] found a positive association with a history of either venous or arterial thrombosis. However, 8 of 11 studies with available results[10,11,15-17,19-21] found a strong correlation between anti-$ß_2$-GPI IgG and aCL IgG positivity. It must be noted that some of the sample sizes upon which that conclusion was based are very small (e.g., sample sizes of one[15] and 5[11]). One other group[20] aside from ours concludes that aCL and anti-$ß_2$-GPI IgG are distinct and independently occurring antibodies. Overall, therefore, the results regarding an association with either thrombosis alone or secondary APS are quite inconclusive. In contrast, only one[19] of the 11 studies that evaluated RPL independently notes an association between the presence of anti-$ß_2$-GPI and RPL in the context of SLE, and while we did note a trend, this was based upon a very small number of patients, and was no higher than the incidence of LAC in those women (Table 3).

Despite the apparent similarity of the selected studies in Table 6, there are inconsistencies that make comparisons difficult. Some investigators, like ourselves, do not divide their patient groups with regard to the current classification criteria for APS. Most simply dichotomize with regard to the presence or absence of thrombosis and antiphospholipid antibodies. Some investigators report antibody prevalences for all patients regardless of their primary diagnosis (e.g., SLE, primary APS, or other connective tissue disease) and then group antibody positive patients based on clinical histories of thrombosis or RPL, without reference to laboratory variables. In most cases, anti-$ß_2$-GPI are measured by independently developed assays and as no standardized sera are available, there are unavoidable, unpredictable interlaboratory variations.

After reviewing the available literature, it is apparent that there is variability among studies in the case selection and definitions used as well as the assay systems employed. It is therefore difficult to determine the significance of this antibody in the clinical context of SLE and whether it is superior to existing tests. Also, within a single center, the occurrence of certain events such as RPL in our patients with SLE may be too uncommon to draw any firm conclusions. There is a need for a multicenter prospective study to investigate the clinical utility of these various antibodies using a clearly defined group of patients with verified clinical outcomes and a standardized set of assays.

We found that antibodies to $ß_2$-GPI were frequently seen

Table 6. Literature review of prevalence of $ß_2$-GPI IgG vs aCL IgG in patients with SLE and clinical associations. Unless noted otherwise, all autoantibodies were evaluated using in-house assays.

Investigator	n SLE	Pos aCL, %	Pos $ß_2$-GPI, %	$ß_2$ + Secondary APS	$ß_2$ + Thrombosis	$ß_2$ + RPL	Pos aCL/Pos $ß_2$-GPI
Bruce, 2000	133	13.5*	15.8*	No	No	No	14/49
Guerin, 1997[15]	20	30.0**	5.0	No	No	No	1/1
Teixido, 1997[16]	79	36.7	6.3	Yes	Yes	No	11/16
Swadzba, 1997[17]	127	42.5	20.4	NA	Yes	No	23/26
Amengual, 1996[18]	81	41.9	40.7	Yes	No	No	All
Tsutsumi, 1996[9]	308	12.3	10.1	NA	Yes	No	NA
Tubach, 2000[10]	102	23.5***	18.6	No	No	No	15/18[23]
Matsuda, 1995[11]	36	38.8	13.9	No	No	No	5/5
Kaburaki, 1995[19]	140	43.6	15.0	Yes	Yes	Yes	All
Cabiedes, 1995[20]	94	45.7	39.4	Yes	Yes	No	19/37
Viard, 1992[21]	47	70.2	36.2	NA	Yes	No	15/17
Horbach, 1996[22]	175	46.9	17.1	NA	No	NA	NA

Antibodies were measured using *Inova, **Diastat, or ***BMD kits. NA: not available.

(35%) in our population with SLE. The prevalence of anti-$ß_2$-GPI is similar in those with and without clinical features of secondary APS. Antibodies to $ß_2$-GPI did, however, identify 6 patients with clinical features of APS who were negative for standard tests, suggesting that in individual cases, anti-$ß_2$-GPI may provide additional information for the classification of APS in SLE. Our literature review revealed that there is still no agreement regarding the significance of anti-$ß_2$-GPI and secondary APS in SLE.

REFERENCES

1. Wilson WA, Gharavi AE, Takao K, et al. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome. Arthritis Rheum 1999;42:1309-11.
2. Roubey RAS. Immunology of the antiphospholipid antibody syndrome. Arthritis Rheum 1996;39:1444-54.
3. Abu-Shakra M, Gladman DD, Urowitz MB, Farewell VT. Anticardiolipin antibodies in systemic lupus erythematosus. Clinical and laboratory correlations. Am J Med 1995;99:624-8.
4. Wahl DG, Guillemin F, de Maistre E, Perret C, Lecompte T, Thibaut G. Risk of venous thrombosis related to antiphospholipid antibodies in systemic lupus erythematosus. Lupus 1997;6: 467-73.
5. Inanc M, Radway-Bright EL, Isenberg DA. $ß_2$-Glycoprotein 1 and anti-$ß_2$-glycoprotein 1 antibodies: where are we now? Br J Rheumatol 1997;36:1247-57.
6. Tan EM, Cohen AS, Fries J, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271-7.
7. Harris EN. Antiphospholipid syndrome. In: Klippel JH, Dieppe PA, editors. Rheumatology. 2nd ed. London: Mosby; 1998:35.1-6.
8. Alarcon-Segovia D, Sanchez-Guerrero J. Primary antiphospholipid syndrome. J Rheumatol 1989;16:482-8.
9. Tsutsumi A, Matsuura E, Ichikawa K, et al. Antibodies to $ß_2$-glycoprotein I and clinical manifestations in patients with systemic lupus erythematosus. Arthritis Rheum 1996;39:1466-74.
10. Tubach F, Hayem G, Marchand J-L, et al. IgG anti-$ß_2$-glycoprotein I antibodies in adult patients with systemic lupus erythematosus: prevalence and diagnostic value for the antiphospholipid syndrome. J Rheumatol 2000;27:1437-43.
11. Matsuda J, Saitoh N, Gotoh M, Gohichi K, Tsudamoto M. Prevalence of $ß_2$-glycoprotein I antibody in systemic lupus erythematosus patients with $ß_2$-glycoprotein I dependent antiphospholipid antibodies. Ann Rheum Dis 1995;54:73-5.
12. Day HM, Thiagarajan P, Ahn C, Reveille JD, Tinker KF, Arnett FC. Autoantibodies to $ß_2$-glycoprotein I in systemic lupus erythematosus and primary antiphospholipid antibody syndrome: clinical correlations in comparison with other antiphospholipid antibody tests. J Rheumatol 1998;25:667-74.
13. Fanopoulos D, Teodorescu MR, Varga J, Teodorescu M. High frequency of abnormal levels of IgA anti-$ß_2$-glycoprotein I antibodies in patients with systemic lupus erythematosus: relationship with antiphospholipid syndrome. J Rheumatol 1998;25:675-80.
14. Sanfilippo SS, Khamashta MA, Atsumi T, et al. Antibodies to $ß_2$-glycoprotein: a potential marker for clinical features of antiphospholipid antibody syndrome in patients with systemic lupus erythematosus. J Rheumatol 1998;25:2131-4.
15. Guerin J, Feighery C, Sim RB, Jackson J. Antibodies to beta-2-glycoprotein I — a specific marker for the antiphospholipid syndrome. Clin Exp Immunol 1997;109:304-9.
16. Teixido M, Font J, Reverter JC, et al. Anti-$ß_2$-glycoprotein I antibodies: a useful marker for the antiphospholipid syndrome. Br J Rheumatol 1997;36:113-6.
17. Swadzba J, De Clerck LS, Stevens WJ, et al. Anticardiolipin, anti-$ß_2$-glycoprotein I, antiprothrombin antibodies and lupus anticoagulant in patients with systemic lupus erythematosus with a history of thrombosis. J Rheumatol 1997;24:1710-5.
18. Amengual O, Atsumi T, Khamashta MA, Koike T, Hughes GRV. Specificity of ELISA for antibody to $ß_2$-glycoprotein I in patients with antiphospholipid syndrome. Br J Rheumatol 1996;35:1239-43.
19. Kaburaki J, Kuwana M, Yamamoto M, Kawai S, Matsura E, Ikeda Y. Clinical significance of phospholipid-dependent anti-$ß_2$-glycoprotein I antibodies in systemic lupus erythematosus. Lupus 1995;4:472-6.
20. Cabiedes J, Cabral AR, Alarcon-Segovia D. Clinical manifestations of the antiphospholipid syndrome in patients with systemic lupus erythematosus associate more strongly with anti-$ß_2$-glycoprotein I than with antiphospholipid antibodies. J Rheumatol 1995;22:1899-906.
21. Viard J-P, Amoura Z, Bach J-F. Association of anti-$ß_2$ glycoprotein I antibodies with lupus-type circulating anticoagulant and thrombosis in systemic lupus erythematosus. Am J Med 1992;93:181-6.
22. Horbach DA, Oort E, Donders RCJM, Derksen RHWM, de Groot PG. Lupus anticoagulant is the strongest risk factor for both venous and arterial thrombosis in patients with SLE. Thromb Haemost 1996;76:916-24.

Editorial

The Spectrum of the Antiphospholipid Syndrome: A Matter of Perspective

For many years, rheumatologists diagnosed what is now known as antiphospholipid syndrome (APS) as systemic lupus erythematosus (SLE). We knew that many of these patients would never manifest other features of SLE but rather demonstrate only thrombosis, thrombocytopenia, or pregnancy loss. As a matter of fact, one or more of those manifestations was likely the reason the patient came to our attention in the first place.

Owing to the work of individuals such as Harris, Hughes, Gharavi, and Alarcon-Segovia[1-5], we have come to appreciate that these patients do not have lupus but rather a separate but related entity, the APS. Although many of us have become complacent with this classification[6], we may still be unclear in many circumstances which patients to treat, when to treat, and how to treat.

APS is first and foremost a clinical disorder. At least one specific laboratory abnormality must be present to classify a patient as APS: either anticardiolipin antibody (IgG or IgM) and/or a lupus anticoagulant (LAC). However, a positive laboratory test is insufficient for the diagnosis. Indeed, there is no rationale in even testing for their presence in the absence of the clinical features[7-11]. When these laboratory markers are determined in a clinically inappropriate situation, the physician is left in a potential quandary: should this asymptomatic patient be treated or is it safe to ignore these laboratory abnormalities?

Although the first question is why the test was ordered in the first place, the more important question is whether the presence of an antiphospholipid antibody (aPL) or LAC is a risk factor for one or more clinical manifestations. The answer to this question directly determines whether treatment is indicated.

The approach to the management of patients with aPL is very much dependent upon the perspective of the attending physician. Although practitioners in other specialties may attend to patients with APS, hematologists, obstetricians, and rheumatologists are most often involved in their management. Faced with a patient with APS or one with merely positive blood work, the management decisions will likely vary among these specialties.

To the hematologist, APS means dealing with hypercoagulability. The patient has a history of venous and/or arterial thrombosis and the goal of therapy is to prevent further thromboembolic events utilizing anticoagulant therapy. APS patients may be adequately treated with low intensity warfarin in the case of venous thrombotic disease or may require high intensity anticoagulation when there is a history of arterial thrombosis[12,13]. In either case, the hematologist sees a patient at risk for recurrent thrombotic events when confronted with APS.

Patients with APS present to the obstetrician with recurrent pregnancy losses (RPL), either early or late, preeclampsia or HELLP syndrome (hemolysis, elevated liver function test, low platelet count), intrauterine growth restriction in the fetus, or placental abruption. Unlike other patients with APS, there may be no history of thromboembolic events. Therapy in these cases is more controversial. Several therapeutic modalities have been investigated and currently heparin in combination with aspirin is the more common treatment option[14,15], although this regimen has recently been challenged[16].

Perhaps the more complex cases with APS present to the rheumatologist. These patients typically have more than one manifestation, including thrombosis, RPL, thrombocytopenia, nephritis, Raynaud's phenomenon, rash such as livedo reticularis, or a vasculitic eruption. Positive antinuclear antibodies may be found suggesting a diagnosis of SLE. Depending upon the clinical picture, one or more different treatment modalities may be used including anticoagulants, aspirin, prednisone, hydroxychloroquine, or intravenous gammaglobulin. In general, the rheumatologist will encounter the patient with multi-

See A study of 75 pregnancies in patients with APS, *page 2025*

ple manifestations more often than either of the above two specialties.

This differential experience of physicians with patients with APS influences the management of all patients classified as APS, regardless of the manifestations. To best illustrate this point, let us examine the problem of recurrent pregnancy loss.

At the 9th International Symposium on Antiphospholipid Antibodies in September 2000, Branch commented on his previous belief that standardized treatment protocols for pregnancy in women with aPL would be available by the end of the 1990s[17]. Indeed, many also thought that the clinical and laboratory criteria for APS had been satisfactorily defined at the 1999 Sapporo Conference[6]. Meanwhile, however, classification criteria and therapeutic protocols have again become controversial after publication of a number of studies highlighting not only the broad spectrum of patients involved — including the paper by Huong, *et al* in this issue[18] — but also the questionable benefit of any therapeutic intervention at all[16].

There have been numerous studies over the last 15 years evaluating the efficacy of acetylsalicylic acid[16], prednisone[19], unfractionated[15] and low molecular weight heparin[20], and most recently intravenous gammaglobulin[21], alone or in various combinations. While many of the studies have been well designed and appropriately powered and analyzed, the varied outcomes are frequently not reproducible at other centers.

In our opinion, based on treating women with APS in our clinic for the past 15 years, the discrepancy in experience with respect to the therapeutic efficacy in the treatment of RPL is likely due to patient heterogeneity, laboratory variability, and the disparate perspectives of the obstetrician, hematologist and rheumatologist.

The example of a 30-year-old woman with a history of 3 pregnancy losses all between 8 and 10 weeks' gestation who has an IgM anticardiolipin antibody of 25 MPL illustrates our point. Current guidelines from the American College of Obstetrics and Gynecology (ACOG) and others[22,23] state that this patient requires treatment with heparin and aspirin. The guidelines do not recommend that further investigations be undertaken, but rather that the positive serology combined with this clinical picture is sufficient to justify intervention. Some obstetricians might recommend further investigation. For example, what if further investigation revealed the presence of a uterine septum? What abnormality would be deemed the more clinically significant? If ACOG guidelines were followed to the letter, the septum would never have been detected, as no anatomic investigations would have been pursued. Our experience in evaluating many patients with first trimester losses has revealed numerous patients with this exact clinical picture.

A hematologist seeing this patient with a history of recurrent miscarriage and aPL would likely follow other guidelines recently published by Ginsberg, *et al*[24], which also recommend treatment for pregnant women with aPL without further investigation. Of course the hematologist would be more concerned that this woman would be at risk for a thromboembolic event, further justifying anticoagulant therapy. Does the fact that this woman has no history of thromboembolism change her risk of a thrombotic event during a future pregnancy? Should this patient be viewed in the same light as a woman with one pregnancy loss at 24 weeks characterized by intrauterine growth restriction and placental infarction, with a history of venous thromboembolism? Depending upon the attending physician's orientation, we submit that in all probability, these two patients may be viewed as having the identical disease with the same risks during a pregnancy.

The question prompted by these two case scenarios relates to treatment. If it is accepted that heparin and aspirin is the appropriate therapy for the treatment of recurrent pregnancy loss in women with an aPL, then both patients should be so treated. However, we believe these patients to be quite different, but representative of the spectrum of APS. One randomized controlled trial has been published that found 85% of women with RPL and an aPL had a successful pregnancy outcome whether treated with aspirin alone or placebo[16]. Our own experience with patients fulfilling the same criteria resulted in a 51% successful outcome[19] without treatment, whereas Rai, *et al* had only a 10% success rate in untreated cases[25]. These 3 studies, with success rates in untreated patients ranging from 10 to 85%, highlight the lack of agreement regarding this population. Perhaps the variation from center to center reproducing results of similar therapeutic modalities is related to the large variation in baseline success rates in the untreated patient population. This variation, in turn, may simply be due to the possibility we are all investigating slightly different subgroups within the spectrum of APS.

Women with RPL and an aPL must be viewed as a diverse population rather than as a single disease entity with equivalent risk requiring identical therapy. We must all appreciate that APS describes a wide spectrum, perhaps requiring formal subclassification[26], and patients must be evaluated and treated individually. Further, treatment must be directed towards clinical disease and not laboratory markers, particularly in light of the continuing controversy regarding the significance of IgM aCL and lower titers of IgG aCL[26]. It is our belief that further controlled clinical trials addressing the problem of recurrent pregnancy loss in women with aPL will serve to emphasize the clinical heterogeneity in this group of patients and support a more rational approach to therapy.

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REFERENCES

1. Harris EN. A reassessment of the antiphospholipid syndrome. J Rheumatol 1990;17:733-4.
2. Hughes GRV. Hughes's syndrome: the antiphospholipid syndrome. A historical view. Lupus 1998;7 Suppl 2:S1-4.
3. Gharavi AE, Pierangeli SS, Levy RA, Harris EN. Mechanisms of pregnancy loss in antiphospholipid syndrome. Clin Obstet Gynecol 2001;44:11-9.
4. Branch DW, Scott JR, Kockenour NK, et al. Obstetric complications associated with the lupus anticoagulant. N Engl J Med 1985;313:152-6.
5. Alarcon-Segovia D, Cabral AR. The concept and classification of anti-phospholipid/cofactor syndrome. Lupus 196;5:364-7.
6. Wilson WA, Gharavi AE, Koike T, et al. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome. Arthritis Rheum 1999;42:1309-11.
7. Lockwood CJ, Romero R, Feinberg RF, Clyne LP, Coster B, Hobbins JC. The prevalence and biologic significance of lupus anticoagulant and anticardiolipin antibodies in a general obstetric population. Am J Obstet Gynecol 1989;161:369-73.
8. Harris EN, Spinnato JA. Should anticardiolipin tests be performed in otherwise healthy pregnant women? Am J Obstet Gynecol 1991;165:1272-7.
9. Taylor M, Cauchi MN, Buchanan RRC. The lupus anticoagulant, anticardiolipin antibodies and recurrent miscarriage. Am J Reprod Immunol 1990;23:33-6.
10. Love PE, Santoro SA. Antiphospholipid antibodies: anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders. Prevalence and clinical significance. Ann Intern Med 1990;112:682-98.
11. Simpson JL, Carson SA, Chesney C, et al. Lack of association between antiphospholipid antibodies and first-trimester spontaneous abortion: prospective study of pregnancies detected within 21 days of conception. Fert Steril 1998;69:814-20.
12. Khamashta MA, Cuadrado MJ, Mujic F, Taub NA, Hunt BJ, Hughes GRV. The management of thrombosis in the antiphospholipid antibody syndrome. N Engl J Med 1995; 332:993-7.
13. Kearon C, Gent M, Hirsh J, et al. A comparison of three months of anticoagulation with extended anticoagulation for a first episode of idiopathic venous thromboembolism. N Engl J Med 1999;340: 901-7.
14. Kutteh WH. Antiphospholipid antibody-associated recurrent pregnancy loss: treatment with heparin and low-dose aspirin is superior to low-dose heparin alone. Am J Obstet Gynecol 1996;174:1584-9.
15. Rai R, Cohen H, Dave M, Regan L. Randomized controlled trial of aspirin and aspirin plus heparin in pregnancy women with recurrent miscarriage associated with phospholipid antibodies (or antiphospholipid antibodies). BMJ 1997;314:253-7.
16. Pattison NS, Chamley LW, Birdsall M, Zanderigo AM, Liddell HS, McDougall J. Does aspirin have a role in improving pregnancy outcome for women with the antiphospholipid syndrome? A randomized controlled trial. Am J Obstet Gynecol 2000; 183:1008-12.
17. Branch DW. Antiphospholipid antibodies and reproductive problems. Clin Obstet Gynecol 2001;44:1.
18. Huong DLT, Wechsler B, Bletry O, Vauthier-Brouzes D, Lefebvre G, Piette JC. A study of 75 pregnancies in patients with antiphospholipid syndrome. J Rheumatol 2001;28:2025-30.
19. Laskin CA, Bombardier C, Hannah ME, et al. Prednisone and aspirin in women with autoantibodies and unexplained recurrent fetal loss. N Engl J Med 1997;337:148-53.
20. Hunt BJ, Doughty HA, Majurndar G, et al. Thromboprophylaxis with low molecular weight heparin (Fragmin) in high risk pregnancies. Thromb Haemost 1997;77:39-43.
21. Branch DW, Peaceman AM, Druzin M, et al. A multicenter, placebo-controlled pilot study of intravenous immune globulin treatment of antiphospholipid syndrome during pregnancy. Am J Obstet Gynecol 2000;182:12-7.
22. Carson SA, Branch DW. Management of recurrent early pregnancy loss. ACOG Practice Bulletin 2001;24:1-12.
23. Derksen RHWM, De Groot PhG, Nieuwenhuis HK, Christiaens GCML. How to treat women with antiphospholipid antibodies in pregnancy? Ann Rheum Dis 2001;60:1-3.
24. Ginsberg JS, Greer I, Hirsh J. Use of antithrombotic agents during pregnancy. Chest 2001;119 Suppl 1:122S-131S.
25. Rai RS, Clifford K, Cohen H, Regan L. High prospective fetal loss rates in untreated pregnancies of women with recurrent miscarriage and antiphospholipid antibodies. Hum Reprod 1995;10:3301-4.
26. Geis W, Branch DW. Obstetric implications of antiphospholipid antibodies: pregnancy loss and other complications. Clin Obstet Gynecol 2001;44:2-10.

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The Value of Testing for Antiphospholipid Antibodies, Other than aCL and LA, in Systemic Lupus Erythematosus Patients with Thrombosis. Athina Theodoridou, Maria L Bertolaccini, Coleen Hamid, Munther A Khamashta, Graham R V Hughes Lupus Research Unit, London, United Kingdom

Several authors have suggested that testing for other antiphospholipid antibodies may help to identify the APS in SLE patients with thrombosis but repeatedly negative for anticardiolipin antibodies (aCL) and/or lupus anticoagulant (LA). To test this hypothesis, we studied 3 groups of SLE patients: 24 female, 2 male, mean age 46±12, all with history of thrombosis (5 venous, 13 arterial and 8 both venous and arterial thrombosis) and positive for aCL and/or LA. (SLE/APS); 26 female, mean age 40±11, with no history of thrombotic events (SLE only) and 25 female, 1 male, mean age 39±11, all with history of thrombotic events (16 venous, 6 arterial and 4 both venous and arterial events) but repeatedly negative for aCL/LA (SLE-thrombosis). aCL and LA were re-tested in all samples. All patients were tested for IgG and IgM anti-β2GPI and anti-prothrombin antibodies (aPT) by in-house ELISAs.

Results. Anti-β2GPI antibodies were present in 15/26 (58%) patients with SLE/APS (12 IgG and 3 IgM) and 0/26 patients with SLE only and SLE-thrombosis. aPT were present in 10/26 (38%) patients with SLE/APS (9 IgG and 1 IgM) and 1/26 (3.8%) patients with SLE only (IgG isotype) and 3/26 (11.5%) SLE-thrombosis patients (2 IgG and 1 IgM isotypes).

Conclusions: Testing for aPL other than aCL and LA in patients with thrombosis but persistently negative for aCL and LA may be helpfull in selective cases. Anti-β2GPI may not be detected in patients negative for aCL.

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Prevalence and Clinical Significance of Antibodies to Protein S in Lupus Patients with Pregnancy Morbidity. Maria L Bertolaccini, Giovanni Sanna, Gonzalo Pacheco, Munther A Khamashta, Graham R V Hughes Lupus Research Unit, London, United Kingdom

Preliminary data has shown that antibodies directed to protein S (anti-PS) might be associated with the antiphospholipid syndrome in patients with SLE. We designed this study to evaluate the prevalence and clinical significance of anti-PS in a group of SLE patients with pregnancy morbidity.

Patients and methods: This study included 119 female SLE (mean age 46.8±11 , mean disease duration 13.7±9.3). Thirty-one women were also diagnosed as having antiphospholipid syndrome. aCL and LA were present in 73 and 30/119 patients, respectively.

Results: Anti-PS were present 39/119 (33%) patients. Patients with pregnancy morbidity presented anti-PS more frequently than the control group (24% vs. 4%, OR 7.9 [95% CI 2.3-28], p=0.0009). When subdividing the patients into subgroups, those with miscarriages and/or fetal death presented anti-PS much more frequently than the controls (24% and 18% vs. 4%, OR 7.6 [95% CI 2.1-27], p=0.0017 and OR 5.4 [95% CI 1.3–22], p=0.021, respectively). Patients with prematurity, preeclampsia and intrauterine growth restriction presented anti-PS more frequently than the control group (36%, 47% and 44% vs. 4%; OR 13.6 [95% CI 2.8–66], p=0.003, OR 21 [95% CI 5–86], p<0.0001 and OR 19 [95% CI 4–99], p=0.0014, respectively).

Conclusions. Anti-PS are frequent in SLE. Although they cannot discriminate within the SLE population, their presence is more common in patients with pregnancy morbidity than in the control group. Further studies including larger populations are needed to elucidate the real value of these antibodies.

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Heart Valve Lesions Related to Antiphospholipd Antibody Syndrome: Value of Transesophageal Echocardiography. Elisa Albuquerque[1], Oscar Ribeiro[1], Sérgio S Xavier[2], Marcia Castier[1], Jose Angelo Papi[2], Denise S Silveira[2], Maria Isabel Dutra[2], Alycia C Fonseca[2], Evandro M Klumb[1], Roger Levy[1] [1]Universidade do Estado do Rio de Janeiro, Rio de Janeiro, Brazil[2]Universidade Federal do Rio de Janeiro, Rio de Janeiro, Brazil

To study the prevalence of cardiac valvular alterations in a population of patients with antiphospholipid syndrome (APS), we examined by transthoracic (TT) and multiplan transesophageal echocardiography (TE-ECHO) 61 patients with APS according to Wilson's criteria (A&R, 1999). Twenty-three patients had primary and 38 had APS secondary to systemic lupus erythematosus. Clinical manifestations and TE-ECHO findings of the 2 groups were compared. The same examiner, with the same equipment, blinded for patients' diagnosis, performed all exams. In 11 of 23 (53%) primary APS patients and 26 of 38 (68%) secondary, valvar alterations were found by TE-ECHO, only one patient had an altered TT-ECHO. The most commonly found alterations were: valvular regurgitation (47.8% in primary group and 52.6% in the secondary); valvar thickening, that occurred frequently in both groups (39% and 34.2%, respectively), valvar vegetations, that were more common in the primary APS group (17.4% and 13.2%, respectively). The mitral valve was most commonly affected, with dysfunction (26% in primary APS and 47.4% in secondary) and thickening (39% and 34.2%), followed by the aortic valve involvement. Vegetations in primary APS were evenly distributed among mitral and aortic valves; while the vegetations found in 5 secondary APS were all restricted to mitral valve. We did not observe ventricular dysfunction in none of the patients with primary APS, while it was found in 13% of the patients with secondary APS. TE-ECHO is a powerful diagnostic tool for evaluating valvular function in both primary and secondary APS.

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Low aCL IgG Titers In Recurrent Pregnancy Loss Signal the Need for Redefining APS. Christine A Clark[1], Karen A Spitzer[1], Jamie N Nadler[2], Carl A Laskin[2] [1]START Reproductive Biology Unit, Toronto, ON, Canada[2]University of Toronto, Toronto, ON, Canada

Objective: To compare the distribution and titres of aCL IgG in women with unexplained early recurrent pregnancy loss (RPL), systemic lupus erythematosus (SLE) or anti-phospholipid syndrome (APS) with a history of thrombosis.

Methods: Results for 280 sera tested for aCL IgG using a commercial kit (INOVA) were selected consecutively (140 positive [>15 GPL], 140 negative [≤15 GPL]). Charts were retrospectively reviewed and diagnoses recorded. Data were analysed using Sigma Stat to compare distribution and titres for each group.

Results: Sera were distributed among the 3 diagnostic categories as follows: RPL 144/280 (51.4%); SLE 83/280 (29.6%); and APS 53/280 (18.9%). Of the 144 RPL sera, 42 (29.2%) were positive; of the 83 SLE sera, 50 (60.2%) were positive; and of the 53 APS sera, 48 (90.6%) were positive for aCL IgG. The prevalence in each group was significantly different from the others ($p \leq 0.0001$). The titres were also significantly different among the 140 positive sera, when divided into diagnostic classifications. The highest titres were seen among patients with APS, and the lowest among patients with RPL (APS vs RPL: $p \leq 0.0001$; APS vs SLE: $p = 0.04$; SLE vs RPL: $p < 0.01$). No women in the RPL group had an aCL value of > 32 GPL, compared to 12/50 (24%) of women in the SLE group and 24/48 (50%) of women in the APS group. In contrast, 36/42 (85.7%) of women in the RPL group had aCL levels between 15–20 GPL, compared to 20/50 (40%) of women in the SLE group and 13/48 (27.1%) of women in the APS group. Using a cut-off of 30 GPL, the aCL test has a sensitivity of 47.2%, a specificity of 99.3% and a positive predictive value of 96.2% for APS vs RPL.

Conclusion: Although about 30% of women with RPL were positive for aCL IgG, their titres were significantly lower than those of women with SLE or APS and none had a titre higher than 32 GPL. Women in the RPL group with a positive aCL >25 GPL and ≥ 3 early losses but no history of thrombosis nevertheless fulfil current classification criteria for APS. Our data indicate that this group appears to comprise a subset within APS with a distinct aCL IgG profile. These women may have a different prognosis requiring a modified therapeutic response.

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Preterm Deliveries in Women with Systemic Lupus Erythematosus

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ABSTRACT. ***Objective.*** To compare the clinical, laboratory, and demographic variables of women in our clinic with systemic lupus erythematosus (SLE) who have had a pregnancy resulting in a live birth and identify any correlations with either term or preterm delivery.

Methods. Pregnancies in women with SLE from 1999 to 2001 were retrospectively reviewed. We recorded demographic data, disease activity (SLE Disease Activity Index, SLEDAI), obstetric history, prednisone dosage, other medications taken during pregnancy, history of renal disease, and autoantibody status [including antinuclear antibody, anti-DNA, anticardiolipin IgG (aCL), and lupus anticoagulant (LAC)]. Preterm delivery was defined as gestational age at delivery < 37 weeks. We performed a literature survey using PubMed and the key words SLE, pregnancy, and outcome.

Results. Of the 72 pregnancies, 28 (38.9%) resulted in preterm deliveries. There were no significant differences in any demographic or disease variables measured comparing term versus preterm delivery groups. More women in the preterm group were taking ≥ 10 mg/day prednisone during their pregnancy (50.0% vs 22.2%; p = 0.028), and the mean dose was significantly higher than the term group taking ≥ 10 mg/day (24.8 vs 16.7 mg/day; p = 0.047). There was a higher prevalence of women with aCL IgG in the preterm group (p = 0.023). The mean weeks gestation was shorter for women positive for aCL IgG compared to the group negative for aCL (34.9 ± 4.4 vs 37.5 ± 3.2 weeks, respectively; p = 0.032). There was no difference in second trimester disease activity between the term and preterm groups (33.3% and 36.4% of each group had a SLEDAI of 0). However, significantly more women in the term group received no medication during their pregnancies compared to women in the preterm group (20.0% vs 0.0%; p = 0.031).

Conclusion. The rates of preterm deliveries, premature rupture of membranes, intrauterine growth restriction, and aPL in SLE pregnancies vary considerably in published reports, most of which are retrospective analyses. Our rates closely approximate the median values for all measures. We found preterm deliveries to be associated with disease activity (as determined by the use of any medication throughout pregnancy vs no medication, and prednisone dose ≥ 10 mg/day) and the presence of aCL IgG but not LAC. Our results suggest that inactive disease rather than controlled disease at the onset of pregnancy may be the determining factor in extending SLE pregnancies to full term, thereby decreasing maternal and fetal morbidity. (J Rheumatol 2003;30:2127–32)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS PREGNANCY
PRETERM DELIVERY ANTICARDIOLIPIN ANTIBODIES
PREDNISONE

The causes of preterm delivery (< 37 weeks' gestation) include premature rupture of membranes (PROM), preeclampsia, the HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets), and preterm labor. The incidence of preterm labor and delivery in women with systemic lupus erythematosus (SLE) varies between 19% and 49% in pregnancies occurring after a diagnosis of SLE compared to the expected rate of 7% for the general population[1-5]. In a large retrospective review of data over a 2 year period, Yasmeen, *et al*[6] found 21.0% of pregnant women with SLE had preterm deliveries (116/555) compared to 4.2% of controls (2520/60,000). This increased frequency of preterm deliveries in SLE has been variously attributed to the following: increased disease activity (monitored by clinical index, serum C3 concentration at first visit, or prednisone dose), a history of renal disease, PROM, hypertension (requiring antihypertensive treatment or elevation of second trimester diastolic blood pressure), preeclampsia, and the presence of antiphospholipid antibodies (aPL)[7-21].

Any attempt to consolidate published results on SLE pregnancies from different centers is frustrated by incomplete or incompatible reporting methodologies, and it is difficult to formulate a consensus regarding frequencies of events and causal relationships when centers apparently have quite discrepant experiences. The highly variable rate

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of preterm delivery in the literature has been quite rightly attributed to differences in patient demographics and subjectively defined clinical and laboratory measures.

We have not previously reported preterm delivery rates in our clinic. In addition to assessing recent pregnancy outcomes in our patients with SLE, we reviewed the pertinent literature and placed our findings in the context of similar reports.

MATERIALS AND METHODS

Patients. We retrospectively reviewed pregnancy outcomes in our clinic for the period 1999–2001 in patients who fulfilled the American College of Rheumatology criteria for the classification of SLE[22]. Preterm delivery was defined as gestational age at delivery of < 37 weeks.

Clinical variables. Age, disease duration at time of pregnancy, and age of disease onset were obtained from patient charts. Disease activity was determined in the second trimester using the SLE Disease Activity Index (SLEDAI)[23]. Obstetrical history included number of pregnancies, live births, spontaneous abortions, stillbirths, and therapeutic abortions. A history of renal disease was determined based on proteinuria > 0.5 g/24 h prior to the pregnancy and biopsy results when available. Prednisone dosage in the second trimester was recorded in addition to any other medications taken during the pregnancy.

Laboratory variables. Our serological analysis included antinuclear antibodies (ANA titer ≥ 1/80, using a HEp-2 substrate), anti-double stranded DNA antibodies (anti-dsDNA by ELISA), anticardiolipin IgG (aCL IgG; Quantalite Kit, Inova, Intermedico, Markham, ON, Canada), the lupus anticoagulant (LAC) measured in plasma by a panel of coagulation tests (Russell's viper venom time, lupus sensitive partial thromboplastin time, kaolin cephalin clotting time, and a dilute prothrombin time confirmed by 1:1 and 4:1 repeat testing with normal plasma), and a routine activated partial thromboplastin time (aPTT).

Statistical analysis. Data were analyzed using the Sigma Stat programme 9 (Version 2.0, SPSS, Chicago, IL, USA). Student's t test and the Mann-Whitney rank-sum test (for data not normally distributed) were used to compare group means or medians, and 95% confidence intervals (CI) were also calculated. Population prevalences were compared using the z test. A p value < 0.05 was considered significant.

Literature review. Using the PubMed database (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) we entered the search words SLE, pregnancy, and outcome and accessed the titles and abstracts from 1992 to 2002. From those, we identified original studies of lupus pregnancies and obtained full articles for those reporting similar clinical outcome criteria for comparison purposes. We attempted to extract from each report the following data: number of live births, rates of preterm deliveries, PROM, intrauterine growth restriction (IUGR), aCL and/or LAC positivity, and conclusions regarding factors that might contribute to preterm deliveries including prednisone usage or dosage, disease activity, and aPL positivity.

RESULTS

Demographic and clinical variables. There were 88 pregnancies in women with SLE in our clinic between 1999 and 2001, 73 of which resulted in a live birth (82.9%). Of the 73 pregnancies, there were 28 (38.4%) preterm deliveries (< 37 weeks). There were no statistically significant differences between the mean ages, disease durations, ages at disease onset, second trimester SLEDAI, or histories of renal disease in the preterm and term delivery groups (Table 1).

Laboratory variables. There was a statistically significant difference in the frequency of aCL IgG-positive patients in the preterm delivery group compared to the term delivery group (55.5% vs 19.4%, respectively; p = 0.023). There were no significant differences with any other autoantibodies measured (Table 1).

Obstetric histories. There were no differences between the obstetrical histories of either the term or preterm delivery groups (Table 2). There were no significant differences in the obstetrical histories of women who spontaneously went into labor or who required medical intervention in the labor and delivery process regardless of the gestational age (data not shown).

Medications in pregnancy. Significantly more women in the preterm delivery group were taking ≥ 10 mg/day prednisone compared to the term delivery group (50.0% vs 22.2%; p = 0.028; Table 3). The mean dose in the preterm group receiving ≥ 10 mg/day was higher than the mean in the comparable term delivery group (28.8 vs 16.6 mg/day; p = 0.047). There were no other significant differences in the frequencies of medications used by the 2 groups.

There were no women in the preterm delivery group who received no medication at all throughout the pregnancy, compared to 9/45 women in the term delivery group (0.0 vs 20.0%, respectively; p = 0.031), despite the lack of significant difference in second trimester disease activity as measured by SLEDAI (Table 1).

Labour and delivery. Fifty women went into labor spontaneously: 44 eventually delivered vaginally and 6 had Cesarean sections (C-sections) due to nonprogressing labor (Table 4). Of those, 33/50 (66.0%) delivered at or beyond 37 weeks' gestation and 17/50 (34.0%) delivered preterm. There was a trend to increased prevalence of aCL IgG in the spontaneous preterm delivery group compared to the spontaneous term delivery group (58.3% vs 24.0%; p = 0.093, data not shown), but because of small sample size, this did not reach significance. There were no statistically significant differences in number of women in each group receiving prednisone, prednisone dosage, or taking no medication at all throughout the pregnancy.

Twenty-three women required medical intervention (induction and/or C-section) in their deliveries (Table 4). Analyzing demographic, clinical, and laboratory results for spontaneous versus induced deliveries, we found no significant differences between the 2 groups (data not shown).

Complications in pregnancy. Table 4 describes the frequency and distribution of complications requiring delivery intervention. Significantly more women in the preterm delivery group were either induced or had a C-section due to high blood pressure, the HELLP syndrome, fetal distress, preeclampsia, proteinuria, IUGR, and decreased amniotic fluid volume combined than in the term delivery group (10/28 vs 5/45; p = 0.023, 95% CI –0.441 to –0.0592, power with $\alpha = 0.05$: 0.617), although there were

Table 1. Demographic, laboratory, and clinical variables.

Variable, Mean ± SD	Preterm Deliveries, n = 28	Term Deliveries, n = 45	p
Age, yrs (range)	30.6 ± 4.0 (22–39)	31.2 ± 3.9 (23–39)	0.536
Disease duration, yrs (range)	8.3 ± 5.6 (1–21)	7.1 ± 5.4 (1–21)	0.354
Age at disease onset (range)	22.2 ± 5.8 (11–32)	24.0 ± 5.6 (12–36)	0.226
2nd trimester SLEDAI (range)	3.1 ± 3.1 (0–12)	2.3 ± 2.8 (0–12)	0.192
History of renal disease (%)	9 (32.1)	13 (28.9)	0.978
Receiving prednisone (%)	19 (67.9)	24 (53.3)	0.329
ANA (%)	24/25 (96.0)	34/39 (87.2)	0.481
Anti-dsDNA (%)	15/22 (68.2)	21/34 (61.8)	0.904
aCL IgG* (%)	10/18 (55.5)	6/31 (19.4)	0.023
LAC (%)	5/16 (31.3)	11/24 (45.8)	0.557
PTT** (%)	6/25 (24.0)	7/40 (17.5)	0.750

* aCL ≥ 15 GPL. **PTT > 37 s.

Table 2. Comparison of reproductive histories of women with term or preterm deliveries. There were no significant differences between the 2 groups for any variable.

Variable	Preterm Deliveries, n = 28	Term Deliveries, n = 45
Previous pregnancies, mean ± SD	1.8 ± 0.9	2.3 ± 1.3
1 spontaneous abortion (%)	4 (14.3)	11 (24.4)
≥ 2 spontaneous abortions (%)	1 (3.6)	4 (8.9)
≥ 1 stillbirth (%)	3 (12.5)	4 (8.9)
No history of adverse obstetrical outcome (%)	15 (53.6)	23 (51.1)

Table 3. Specific medications used throughout 72 pregnancies of women with SLE. Many women received a combination of therapies.

Medication during Pregnancy	Term Deliveries, n = 45 (%)	Preterm Deliveries, n = 28 (%)	p
Prednisone	24 (53.3)	19 (67.9)	NS
Prednisone dose > 10 mg/day	10 (22.2)	14 (50.0)	0.028
Azathioprine	3 (6.7)	5 (17.9)	NS
Hydroxychloroquine/chloroquine	10 (22.2)	4 (14.3)	NS
Dexamethasone	1 (2.2)	1 (3.6)	NS
LMW heparin	8 (17.7)	5 (17.9)	NS
Aspirin	25 (55.5)	16 (57.1)	NS
IV gamma globulin	1 (2.2)	0 (0)	NS
Aspirin only	4 (8.8)	3 (10.7)	NS
No therapy	9 (20.0)	0 (0.0)	0.031

LMW: low molecular weight.

no significant differences in the frequencies of each individual complication between the term and preterm groups.

Literature review. Using the search words SLE, pregnancy, and outcome, the PubMed database identified 180 publications from 1992 to 2002. From the abstracts, we selected 27 publications and obtained full texts for each. We then selected only observational studies (retrospective or prospective) with adequate sample size that reported outcome data, and measured the same variables for comparison (Table 5).

With a median sample size of 67 and a total of 638 pregnancies, 10 studies including our own found a median of 33.4% (range 7.8%–63.2%) of SLE pregnancies resulted in preterm deliveries. Rates of PROM and IUGR ranged from 5.6% to 37.9% and 2% to 39.5%, respectively, although the medians for both were quite low: 7.5% and 9.4%, respectively. The prevalence of aPL in the different samples ranged from 13.5% to 51.7%, median 32.6%. The median live-birth rate for SLE pregnancies in 10 studies over the last 10 years is 80.9% (mean ± SD 80.1 ± 8.1) Our results closely match the median values for all variables (Table 5).

There was no consensus regarding the influence of disease activity, prednisone usage, or aPL positivity on the incidence of prematurity in SLE pregnancies.

DISCUSSION

In any discussion of preterm deliveries, it is essential to differentiate between those resulting from spontaneous

Table 4. Delivery data for all patients. There was no statistically significant difference in the proportion of women delivering spontaneously in either the term or preterm delivery groups. However, significantly more women in the preterm delivery group were either induced or had a C-section due to high blood pressure, HELLP syndrome, fetal distress, preeclampsia, proteinuria, IUGR, and decreased amniotic fluid than in the term delivery group (10/28 vs 5/45; p = 0.026, 95% CI 0.0554 to 0.437, power with $\alpha = 0.05$: 0.602).

Labor and Delivery	Total n = 73 (%)	Preterm Deliveries, n = 28 (%)	Term Deliveries, n = 45 (%)
Spontaneous onset of labor	50 (68.5)	17 (60.7)	33 (73.3)
Normal vaginal delivery	44 (60.3)	15 (53.6)	29 (64.4)
C-section (nonprogressing labor)	6 (8.2)	2 (7.1)	4 (8.9)
Prelabor intervention: C-section	14 (19.2)	8 (28.6)	6 (13.3)
Breech presentation	3 (4.1)	1 (3.6)	2 (4.4)
High blood pressure	2 (2.7)	2 (7.1)	0
HELLP	1 (1.4)	1 (3.6)	0
Preeclampsia	2 (2.7)	1 (3.6)	1 (2.2)
IUGR	2 (2.7)	2 (7.1)	0
Fetal distress	2 (2.7)	1 (3.6)	1 (2.2)
Low-lying placenta	1 (1.4)	0	1 (2.2)
Repeat	1 (1.4)	0	1 (2.2)
Prelabor intervention: induction	9 (12.3)	3 (10.7)	6 (13.3)
Decreased amniotic fluid volume	4 (5.5)	2 (7.1)	2 (4.4)
Proteinuria	2 (2.7)	1 (3.6)	1 (2.2)
Miscellaneous*	3 (4.1)	0	3 (6.7)

* Includes post-term inductions.

Table 5. Comparison of results from different studies of SLE pregnancies. Total pregnancies do not include those electively terminated.

Study	Total Pregnancies, n (Live Birth Rate, %)	Index Pregnancies, n	Preterm Deliveries, n (%)	PROM, n (%)	IUGR, n (%)	+ aPL* (%)	Concluded that Prematurity Correlates With		
							Disease Activity	Prednisone Use	aPL
Le Thi Huong[12]	94 (80.9)	76	48 (63.2)	ND	30 (39.5)	10/74 (13.5)	Yes	Yes	No
Lima[14]	108 (82.4)	89	38 (42.3)	5** (5.6)	30 (33.7)	25/73 (34.2)	No	No	No
Johnson[19]	NA***	58	27 (46.6)	22 (37.9)	ND	ND	No	No	No
Le Thi Huong[18]	60 (80.0)	48	29 (60.4)	ND	1 (2.0)	Not Clear	No	No	Yes
Rahman[16]	121 (71.1)	86	21 (24.4)	ND	6 (7.0)	15/29 (51.7)	No	No	No
Carmona[13]	57 (93.0)	53	11 (20.8)	4 (7.5)	5 (9.4)	16 (30.2)	No	No	No
Kobayashi[17]	74 (89.2)	66	11 (16.7)	8 (12.1)	14 (21.2)	12/33 (36.7)	Yes	Yes	No
Georgiou[15]	56 (69.6)	39	3 (7.8)	ND	2 (5.1)	7 (17.9)	Yes	ND	No
Cortez-Hernandez[11]	95 (71.6)	68	19 (27.9)	ND	21 (31.0)	22/68 (32.4)	Yes	Yes	Yes
Present study	88 (83.0)	73	28 (38.4)	5 (6.8)	2 (2.7)	16/49 (32.7)	Yes	Yes	Yes
Median (min–max)	88 (80.9)	67 (39–89)	33.4% (7.8–63.2)	7.5% (5.6–37.9)	9.4% (2–39.5)	32.6% (13.5–51.7)	5/10 Yes	4/9 Yes	3/10 Yes

* Anticardiolipin IgG and/or IgM and/or LAC. ** The authors reported both 5.6% in the results section and 11% in the discussion section. *** Only reported data for women with gestations > 23 weeks. ND: not done or not discussed.

onset of labor before 37 weeks and those that are the result of a clinical condition, either maternal or fetal, necessitating medical intervention. In our sample of women with SLE, 34.0% (17/50) went into labor before 37 weeks' gestation spontaneously, not a significantly different proportion than the proportion in the whole sample including both spontaneous and induced deliveries (38.4%, 28/73; p = 0.759; Table 4).

We found 38.4% of deliveries were preterm in our sample of 73 SLE pregnancies. They were associated with disease activity as determined by either prednisone dose or no medication at all, and aCL IgG positivity. However, after controlling for spontaneous versus induced onset of labor, these differences disappeared other than a trend toward increased prevalence of aCL IgG in the preterm group. There were no differences in the demographic or obstetrical histories of our patients to indicate any associations with preterm delivery.

The increased incidence of preterm deliveries in SLE compared to the general population is well described, and a number of factors have been reported in association with the increase including hypertension, active disease at pregnancy

outset, decreased serum C3, prednisone treatment, and aCL[7,9,10]. However, there is no consensus regarding these factors (Table 5). Johnson, *et al*[19] and Lima, *et al*[14] did not find disease activity, prednisone use, or serologic studies predictive of preterm delivery. In contrast, in a review of 9 years' experience at the Hopkins Lupus Pregnancy Center, Petri reported that disease activity, measured by either disease activity indices or laboratory markers, was predictive of preterm deliveries in SLE patients[20].

Lockwood, *et al*[24] reported an association between anticardiolipin antibodies and preterm delivery in a general obstetric population. Anticardiolipin antibodies were associated with an increased incidence of preterm deliveries in a report by Ramsey-Goldman, *et al*[8]. However, in that study, preterm delivery was defined as before 38 weeks' vs 37 weeks' gestation, and pregnancies were stratified by timing before and after diagnosis of SLE and by number of pregnancies, before the increase became significant. Cortes-Hernandez, *et al*[11] also noted a significant association of preterm delivery with aPL positivity, using multiple logistic regression. Others have not found an association with aPL[14,20]. Although aCL were found twice as frequently in the preterm delivery group, there did not appear to be a coincident occurrence of IUGR or placental abnormalities that are characteristic of the aPL syndrome. Interestingly, there was no increase in the frequency of LAC in the preterm group. There was no distinguishing autoantibody profile, other than the increased aCL, associated with preterm delivery.

In a randomized controlled trial, we found a significant increase in preterm delivery but not PROM or IUGR in a group of women with recurrent pregnancy loss and circulating autoantibodies (but not SLE) treated with prednisone and acetylsalicylic acid, suggesting that prednisone itself may have a significant effect on the incidence of preterm delivery[25]. We did not find an increased number of women using prednisone in the preterm delivery group in this study of SLE pregnancies, but we did find a significantly higher number of women in the preterm delivery group taking ≥ 10 mg/day. This was not a reflection of increased disease activity during the pregnancy as measured by the second trimester SLEDAI, as we found no association between disease duration or activity with preterm delivery. The higher frequency was more likely due to a higher antenatal dose maintained throughout the pregnancy, reflecting a situation in which the patients had stable but not inactive disease at onset of pregnancy. There are a number of investigators whose experience differs from ours, although others have also observed this association between prednisone dose and preterm delivery[11,12,20].

An increase in the frequency of renal disease in SLE patients with preterm deliveries has been reported[13], but we did not observe this in our sample.

Our study has some limitations and our results should be interpreted within the context of those limitations. Because the study was retrospective, we have incomplete laboratory results (Table 1) and the sample sizes for each antibody measured are variable, resulting in inadequate or reduced confidence in our statistical analysis. In addition, the SLEDAI is validated for prospective studies and may be limited when applied retrospectively[26]. For example, 20% of women in the term delivery group were medication-free during their pregnancies compared to none of the women with preterm deliveries, although there was no significant difference in the mean disease activity levels between the 2 groups as determined by our chart review. Intuitively, one would expect to find higher levels of disease activity in conjunction with higher doses of prednisone, and we did not find this to be the case. Whether the timing of the SLEDAI assessment would alter this result is not known.

With regard to the literature review, it was challenging to extract the necessary values from many of the reports, and in several cases we had to estimate rates and prevalences from raw data scattered throughout the results and discussion sections. In addition, there are no standardized criteria for classifying patients, symptoms, and laboratory values. There is continuing controversy regarding assay methodology and titer interpretation of aCL levels, and reporting of aPL positivity may not specifically provide aCL or LAC results separately. This makes comparisons all the more difficult and unreliable. Our attempt to compare specific outcomes and frequencies of preterm deliveries, PROM, IUGR, and aPL positivities in SLE pregnancies in different reports highlighted the difficulty of developing a consensus in the absence of standardized documentation.

On a more positive note, and in conclusion, it should be reiterated that 73 of 88 pregnancies among our patients with SLE over a 2 year period resulted in a live birth (83%) regardless of disease status and therapy. In our experience, and in that of many other investigators, closely monitored SLE pregnancies have a good prognosis for both mother and neonate.

REFERENCES

1. Devoe LD, Taylor RL. Systemic lupus erythematosus in pregnancy. Am J Obstet Gynecol 1979;135:473-9.
2. Lockshin MD, Reinitz E, Druzin ML, Murrman M, Estes D. Lupus pregnancy: case-control prospective study demonstrating absence of lupus exacerbation during or after pregnancy. Am J Med 1984;77:893-8.
3. Mintz G, Niz J, Gutierrez G, Garcia-Alonso A, Karchmer S. Prospective study of pregnancy in systemic lupus erythematosus. Results of a multidisciplinary approach. J Rheumatol 1986;13:732-9.
4. Nossent HC, Swaak TJG. Systemic lupus erythematosus. VI. Analysis of the interrelationship with pregnancy. J Rheumatol 1990;17:771-6.
5. Wong K, Chan R, Lee C. Outcome of pregnancy in patients with systemic lupus erythematosus. Arch Intern Med 1991;151:269-73.
6. Yasmeen S, Wilkins EE, Field NT, Sheikh RA, Gilbert WM. Pregnancy outcomes in women with systemic lupus erythematosus.

J Matern Fetal Med 2001;10:91-6.
7. Wechsler B, Le Thi Huong D, Piette J-C. 1998 update on pregnancy in lupus patients. New horizons, new hopes. Rev Rhum [Engl Ed] 1998;65:619-24.
8. Ramsey-Goldman R, Kutzer JE, Kuller LH, Guzick D, Carpenter AB, Medsger TA. Pregnancy outcome and anti-cardiolipin antibody in women with systemic lupus erythematosus. Am J Epidemiol 1993;138:1057-69.
9. Mok CC, Wong RWS. Pregnancy in systemic lupus erythematosus. Postgrad Med J 2001;77:157-65.
10. Meng C, Lockshin M. Pregnancy in lupus. Curr Opin Rheumatol 1999;11:348-51.
11. Cortes-Hernandez J, Ordi-Ros J, Paredes F, Casellas M, Castillo R, Vilardell-Tarres M. Clinical predictors of fetal and maternal outcome in systemic lupus erythematosus: a prespective study of 103 pregnancies. Rheumatology Oxford 2002;41:643-50.
12. Le Thi Huong D, Wechsler B, Piette J-C, Bletry O, Godeau P. Pregnancy and its outcome in systemic lupus erythematosus. QJM 1994;87:721-9.
13. Carmona R, Font J, Cervaera R, Munoz F, Cararach V, Balasch J. Obstetrical outcome of pregnancy in patients with systemic lupus erythematosus. A study of 60 cases. Eur J Obstet Gynecol Reprod Biol 1999;83:137-42.
14. Lima F, Buchanan NMM, Khamashta MA, Kerslake S, Hughes GRV. Obstetric outcome in systemic lupus erythematosus. Semin Arthritis Rheum 1995;25:184-92.
15. Georgiou PE, Polii EN, Katsimbri P, Sakka V, Drosos AA. Outcome of lupus pregnancy: a controlled study. Rheumatology Oxford 2000;39:1014-9.
16. Rahman P, Gladman DD, Urowitz MB. Clinical predictors of fetal outcome in systemic lupus erythematosus. J Rheumatol 1998;25:1526-30.
17. Kobayashi N, Yamada H, Kisheda T, et al. Hypocomplementemia correlates with intrauterine growth retardation in systemic lupus erythematosus. Am J Reprod Immunol 1999;42:153-9.
18. Le Thi Huong D, Wechsler B, Vauthier-Brouzes D, et al. Outcome of planned pregnancies in systemic lupus erythematosus: a prospective study on 62 pregnancies. Br J Rheumatol 1997;36:772-7.
19. Johnson MJ, Petri M, Witter FR, Repke JT. Evaluation of preterm delivery in a systemic lupus erythematosus pregnancy clinic. Obstet Gynecol 1995;86:369-9.
20. Petri M. Hopkins lupus pregnancy center: 1987-1996. Rheum Dis Clin North Am 1997;23:1-13.
21. Petri M, Allbritton J. Fetal outcome of lupus pregnancy: a retrospective case-control study of the Hopkins Lupus Cohort. J Rheumatol 1993;20:650-6.
22. Tan EM, Cohen AS, Fries J, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271-7.
23. Bombardier C, Gladman DD, Urowitz MB, Carron D, Chang CH. Derivation of the SLEDAI. A disease activity index for lupus patients. The Committee on Prognosis Studies in SLE. Arthritis Rheum 1992;35:630-40.
24. Lockwood CJ, Romero R, Feinberg RF, et al. The prevalence and biological significance of lupus anticoagulant and anticardiolipin antibodies in a general obstetric population. Am J Obstet Gynecol 1989;161:369-73.
25. Laskin CA, Bombardier C, Hannah ME, et al. Prednisone and aspirin is not an effective treatment in women with autoantibodies and unexplained recurrent fetal loss: The results of a double-blind, randomized controlled trial. N Engl J Med 1997;337:148-53.
26. Arce-Salinas A, Cardiel MH, Guzman J, Alcocer-Varela J. Validity of retrospective disease activity assessment in systemic lupus erythematosus. J Rheumatol 1996;23:846-9.

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12

The Critical Role of Arginine Residues in the Binding of Human Monoclonal Antiphospholipid Antibodies to β2Glycoprotein I. Ian Giles[1], Nancy Lambrianides[1], Yiannis Ioannou[1], David Latchman[1], Pojen Chen[2], Reginald Chukwuocha[2], David Isenberg[1], Anisur Rahman[1]. [1]University College London, London, United Kingdom; [2]Department of Medicine, Division of Rheumatology, University of California, Los Angeles, CA

Purpose: Previously, we reported that specific arginine (Arg) residues in the heavy chain (VH) of a human pathogenic β2GPI-dependent antiphospholipid antibody (aPL), IS4, and light chain (VL) of a human anti-DNA antibody, B3, were important in conferring their ability to bind cardiolipin (CL). We were unable to demonstrate binding to β2GPI, due to the lower yield of antibody in the transient expression system. In order to improve the yield of IgG, a stable expression system was developed to examine the importance of specific Arg residues in IS4VH and paired VL in binding to native β2GPI and to domain I (D1) of β2GPI alone,which we hypothesised to contain the immuodominant epitopes for aPL.

Methods: VH and VL cDNA were cloned into the same expression vector containing appropriate constant region cDNA. The distribution of Arg residues in complementarity determining regions (CDRs) of VH and VL sequences were altered by site-directed mutagenesis or VL exchange. Three 2a2 derived VL sequences (IS4, B3 and UK4, a β2GPI-independent aPL) were paired with native IS4VH. Five variants of IS4VH, containing different patterns of Arg residues in CDR3, were paired with IS4VL and three with B3VL. These plasmids, containing the dihydrofolate reductase gene (*dhfr*), were transfected into CHO*dhfr*$^-$ cells by electroporation. To select for incorporation of the plasmid, transfected cells were grown in medium lacking ribonucleosides and deoxyribonucleosides. Individual colonies of cells were isolated and, once confluent, the cell supernatants were tested for the presence of whole IgG by ELISA. Plasmids were amplified by exposure to methotrexate. The ability of the 11 expressed VH/VL combinations to bind CL, β2GPI and D1 were determined by direct ELISA. Recombinant D1 was produced by a novel bacterial expression system developed by our group and contains a C-terminal his-tag to facilitate purification and binding to nickel chelate ELISA plates.

Results: Of four Arg residues in IS4VH CDR3 substituted to serines, two at positions 100 and 100g had a major influence on the strength of CL, β2GPI and D1 binding whilst two at positions 96 and 97 had no effect on CL binding but had a moderate effect on β2GPI and D1 binding. The presence of B3VL conferred the strongest binding to CL, β2GPI and D1. IS4VH/UK4VL however exhibited good binding to D1, very weak CL binding and no binding to β2GPI.

Conclusion: An increased yield of IgG from a stable expression system has confirmed that similar subsets of Arg residues at specific locations in the CDRs of VH and VL of pathogenic aPL are important in determining their ability to bind β2GPI and D1 alone, as well as CL. The similar patterns of binding to β2GPI and D1 obtained with the majority of the VH/VL combinations tested suggests that the epitope for these antibodies resides upon domain I of β2GPI.

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13

A Novel, Efficient Prokaryotic Expression System of Domain I of Human Beta2 Glycoprotein I. Yiannis Ioannou, Nancy Lambrianides, Ian Giles, David Latchman, David Isenberg, Anisur Rahman. University College London, London, United Kingdom

Purpose: Expression of eukaryotic proteins in bacteria, though often the expression method of choice, is frequently hampered by a number of properties that make expression difficult. Consequently other more expensive and time consuming methods of production in higher systems need to be explored. Domain I (D1) of human beta2 glycoprotein I (β2GPI) is thought to contain crucial antibody binding epitopes for antiphospholipid antibodies (aPL), which are critical to the pathogenesis of the antiphospholipid syndrome (APS). D1 expression has been established using baculovirus in insect cells and has recently been studied therapeutically for its use in APS as a tolerogen. We hypothesised that the presence of 'minor' codons in the native cDNA sequence of D1, as well as disulphide bonds and bacterial toxicity were the main obstacles accounting for the current absence of a prokaryotic expression system of D1. Hence our aim was to establish the first expression system of D1 of human β2GPI in *E.coli* by addressing the factors identified in our hypothesis.

Methods: Using recursive PCR, a synthetic gene incorporating 'major' codons preferred by bacteria was made encoding for a his-tagged D1 protein with an N-terminal signal peptide. This was cloned into an expression plasmid pET(26b) (Invitrogen), with expression driven by the T7*lac* promoter in protease deficient BL21(DE3) *E.coli*. By virtue of the signal sequence, periplasmic localisation of D1 aided disulphide bond formation, which was subsequently purified via nickel chromatography utilising the C-terminal his-tag. Toxicity was addressed by tightly regulating expression through additional *lac* repression at the plasmid level provided by the high stringency T7*lac* promoter. In addition cultures were incubated in the presence of glucose at 30°C.

Results: Purified, soluble D1 in yields of 750μg/L bacterial culture were obtained. Correct folding was confirmed on Western blot by binding to two murine monoclonal anti-D1 antibodies (6C4C10 and mAb-16) that recognise conformational epitopes of D1. Binding to an affinity purified human monoclonal aPL (IS4) was confirmed by direct immunoassay. Expression using the native human cDNA sequence of D1 in the same construct under identical conditions yielded 35% less his-tagged D1 compared to the recombinant sequence optimised for bacteria.

Conclusions: This is the first description of prokaryotic expression of soluble D1 of β2GPI at good levels. Ease, efficiency and relatively inexpensive cost of this production system will aid future production and studies of D1, helping to further elucidate its role as an antigenic target in the pathogenesis of APS. Furthermore this problem-based approach may potentially be applied to other eukaryotic proteins containing disulphide bonds, which do not require any additional post-translational modification, and where prokaryotic expression in the past may have proved difficult.

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14

Screening Results for 847 Women Attending a Recurrent Pregnancy Loss Clinic. Christine A. Clark[1], Carl A. Laskin[1], Jeffrey S. Ginsberg[2], Mark A. Crowther[2], Karen A. Spitzer[1], John C. Kingdom[3], Jon F.R. Barrett[3], Michael Gent[2]. [1]University of Toronto, START Reproductive Biology Unit, Toronto, ON, Canada; [2]McMaster University Medical Centre, Hamilton, ON, Canada; [3]University of Toronto, Toronto, ON, Canada

Purpose: To present the screening results of a large ethnically heterogeneous population of 847 women with a history of unexplained recurrent pregnancy loss (RPL) referred to a tertiary clinic and determine the distribution of etiologies.

Methods: Data regarding age, obstetric history, autoantibody and thrombophilia positivity, and hormonal, genetic and anatomic abnormalities were prospectively obtained during screening for the HepASA trial. Laboratory analysis included: antinuclear antibody (ANA); anti-ds and ss DNA and anti-lymphocyte IgM antibody; anti-cardiolipin (aCL) IgG and IgM; a lupus anticoagulant (LAC) panel; and a thrombophilia screen comprising protein C, protein S, antithrombin III, and factor V Leiden. Women were also investigated for the presence of anatomic, genetic and hormonal abnormalities.

Results: The mean age of the screened population was 34.9 ± 4.7 years. Forty-seven (5.5%) patients screened denied a history of consecutive losses. Six hundred and sixty-five women (78.5%) had ≥ 2 consecutive losses before 14 weeks' gestation; 27 women (3.2%) had ≥ one late loss (>14 weeks' gestation); and 108 women (12.8%) had a history of both early and late losses. ANA, anti-DNA and anti-lymphocyte antibodies were found in 109 (12.9%); at least one anti-phospholipid antibody (including aCL and LAC) were found in 133 (15.7%); and a thrombophilia defect was found in 63 (7.4%). 286 women (35.8%) presented with at least one autoimmune or coagulation abnormality. 99 women (12.4%) had an anatomic, hormonal or genetic abnormality and were sent for appropriate treatment or counselling. There were no significant differences in the ages of the various groups.

Conclusions: We found a low prevalence of inherited thrombophilias (7.4%) in our large, ethnically heterogeneous sample, contrary to earlier reports in smaller, ethnically homogeneous populations. Recurrent early loss represented the most common obstetric history; late loss only occurred in a very small minority of women (3.2%). Slightly more than 10% of women had a genetic, anatomic or hormonal abnormality that might account for their

RPL. About 35% of women had either an autoimmune or a coagulation abnormality towards which most treatment protocols are currently directed. Almost 60% of women presenting at our clinic with a history of RPL, however, had no identifiable putative etiology for their condition.

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15

Frequency of Anticoagulated Patients Meeting Criteria for APS. Richard H. White[1], Andrew Dunn[2], Peter Kaboli[3]. [1]UC Davis, Sacramento, CA; [2]Mt Sinai, New York, NY; [3]Univ Iowa, Iowa City, IA

Purpose: Although there are specific criteria for diagnosis of the antiphospholipid antibody syndrome (APS), most physicians are not aware that these criteria include repeat testing, documenting the presence of either a lupus anticoagulant or medium to high levels of anticardiolipin antibody. An incorrect diagnosis of APS potentially results in unnecessary long-term oral anticoagulation.

Methods: A cross-sectional study at three university-based anticoagulation clinics was performed. All patients diagnosed as having APS and being treated with warfarin were identified. Levels of anticardiolipin antibody were classified as low-positive if abnormal but less than 40 GPL/MPL units and high-positive if ≥40 units. Strength of meeting the Sapporo diagnostic criteria was graded as definite, possible, and not meeting criteria.

Results: Of 103 cases, 97 had clinical and laboratory data available. Only 10 cases (10%, 95% Confidence Interval 5–19) had a definite diagnosis of APS, 16 (16%, 10–26) had a possible diagnosis, and 71 (73%, 63–81) did not meet the criteria. Among 91 cases tested for anticardiolipin antibody or the lupus anticoagulant (LA), only 35 (39%, 95% CI = 29–49%) had either one or more positive LA test or two repeatedly high-positive anticardiolipin antibody levels, and only 50 (55%, 95% CI = 44–65%) had one or more high-positive anticardiolipin antibody levels or a positive lupus anticoagulant test. The proportion of cases meeting the Sapporo criteria was similar at the three sites: 5%, 13%, and 13% of cases. Repeat laboratory testing was performed in only 49 cases (51%, 40–61).

Conclusions: Only a small percentage of patients being treated for APS met the Sapporo criteria. Abnormal levels of anticardiolipin antibody were frequently only in the low-positive range, and repeat testing was frequently absent. A quality improvement program that includes expert review of cases referred for chronic anticoagulation care is recommended to ensure appropriate diagnostic testing and treatment of patients with suspected APS.

Disclosure: R.H. White, None; A. Dunn, None; P. Kaboli, None.

16

Factor XII Autoantibodies as a Novel Marker for Thrombosis in Patients with Systemic Lupus Erythematosus. Kirti Mepani[1], Maria Laura Bertolaccini[1], Giovanni Sanna[2], Graham RV Hughes[1], Munther A Khamashta[1]. [1]Lupus Research Unit, The Rayne Institute, St Thomas' Hospital, London, United Kingdom; [2]Rheumatology Department, Homerton University Hospital, London, United Kingdom

It has recently been reported that the presence of antiphospholipid antibodies induces factor XII reduction, and that anti-factor XII antibody (aFXII) can be detected in thrombotic patients. We designed this study to assess the prevalence and clinical significance of aFXII in a large cohort of SLE patients.

Patients and methods: This study comprised 127 patients, all fulfilling at least 4 of the 1982 criteria for the classification of SLE. All patients were tested for IgG and IgM aFXII by an in-house ELISA. One hundred and twenty-three healthy donors comprised the control group.

Results: The study group comprised 123 female with a mean age 42 ± 12 years and a mean disease duration 12.6 ± 8.5 years. Forty-three patients had a history of thrombosis (22 arterial, 11 venous and 13 both arterial and venous) but only 22 fulfilled 1999 Sapporo criteria for APS. Fifty out of the 127 SLE patients (39%) and 9/123 healthy controls (7%) were positive for aFXII (36 IgG, 3 IgM and 11 both from the SLE group and 5 IgG and 4 IgM in the control group). Patients with thrombosis presented IgG and IgM aFXII more frequently than controls (35% vs. 4%, OR 12.6 [95% CI 4.3–37], $P <$ 0.0001 and 13% vs. 3%, OR 4.5 [95%CI 1.2–13], $P = 0.03$, respectively). Levels of IgG and IgM aFXII were also higher in patients with thrombosis than in the control group (13.4 ± 13 vs. 7.6 ± 3, $P < 0.0001$ and 2.0 ± 1.8 vs. 1.5 ± 0.01, $P = 0.005$; respectively). IgG and IgM aFXII were more frequent in patients with arterial thrombosis than in controls (37% vs. 4%, OR 14 [95% CI 4.5–43], $P < 0.0001$ and 14% vs. 3%, OR 5 [95%CI 1.2–20], $P = 0.03$, respectively). Levels of IgG and IgM aFXII were also higher in patients with arterial thrombosis than in the controls (13.8 ± 13 vs. 7.6 ± 3, $P < 0.0001$ and 2.1 ± 2 vs. 1.5 ± 0.01, $P = 0.001$; respectively). IgG but not IgM aFXII were more frequent in patients with venous thrombosis than in controls (42% vs. 4%, OR 17 [95% CI 5–56], $P < 0.0001$ and 12% vs. 3%, $P = 0.09$, respectively).

Conclusion: aFXII are frequent in SLE patients. Their presence is associated with thrombosis making these antibodies a novel potential marker for the APS.

Disclosure: K. Mepani, None; M. Bertolaccini, None; G. Sanna, None; G. Hughes, None; M. Khamashta, None.

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Factor XII Autoantibodies as a Novel Marker for Adverse Obstetric History in Systemic Lupus Erythematosus. Kirti Mepani[1], Maria Laura Bertolaccini[1], Giovanni Sanna[2], Graham RV Hughes[1], Munther A Khamashta[1]. [1]Lupus Research Unit, The Rayne Institute, St Thomas' Hospital, London, United Kingdom; [2]Rheumatology Department, Homerton University Hospital, London, United Kingdom

Factor XII deficiency has been associated with fetal loss. As the presence of antiphospholipid antibodies may induce factor XII reduction, and anti-factor XII antibodies (aFXII) can be detected in patients with fetal loss, we designed this study to assess the prevalence and clinical significance of aFXII in a large cohort of SLE patients with adverse obstetric history.

Patients and methods: This study comprised 83 females with a mean age of 47 ± 11 years and a mean disease duration of 14 ± 9 years, all fulfilling at least 4 of the 1982 criteria for the classification of SLE. IgG and IgM aFXII were measured by an in-house ELISA. One hundred and twenty-three healthy donors comprised the control group.

Results: Thirty-five out of the 83 patients (42%) had adverse obstetric history. Twenty patients had a history of miscarriages $<10^{th}$ week of gestation, 5 had history of fetal deaths $\geq 10^{th}$ week of gestation and 10 had history of both miscarriages and fetal deaths. Only 18 fulfilled 1999 Sapporo criteria for APS. Thirty-three out of the 83 SLE patients (40%) and 9/123 healthy controls (7%) were positive for aFXII (24 IgG, 2 IgM and 7 both from the SLE group and 5 IgG and 4 IgM in the control group). Patients with adverse obstetric history presented IgG but not IgM aFXII more frequently than controls (40% vs. 4%, OR 16 [95% CI 5.1–48], $P < 0.0001$ and 9% vs. 3%, $P = 0.2$, respectively). IgG aFXII were also more frequent in patients with miscarriages and fetal death than in controls (47% and 40% vs. 4%, OR 21 [95% CI 7–65], $P < 0.0001$ and OR 16 [95%CI 4–61], $P = 0.0002$, respectively). The prevalence of IgM aFXII was not different between groups (10% for miscarriages, 13% for fetal death and 3% for the control group). When considering only patients who fulfil Sapporo criteria, IgG but not IgM aFXII were more frequently found in patients with pregnancy morbidity than in the control group (39% vs. 4%, OR 15 [95%CI 4.1–55], $P < 0.0001$ and 11% vs. 3%, $P = 0.2$, respectively).

Conclusion: aFXII, particularly of the IgG isotype, are frequent in SLE patients with adverse obstetric history. Their association with pregnancy morbidity warrants further investigation.

Disclosure: K. Mepani, None; M. Bertolaccini, None; G. Sanna, None; G. Hughes, None; M. Khamashta, None.

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Anti-Cd40 Antibodies in Antiphospholipid Syndrome and Systemic Lupus Erythematosus. Panayiotis Vlachoyiannopoulos, Clio Mavragani, Anthi Balitsari, Efi Bourazopoulou, John Routsias. Medical School, University of Athens, Athens, Greece

Purpose: Anti-β2glycoprotein I antibodies induce the precoagulant activity of the endothelium by cross-linking the β2 glycoprotein (β2GPI) on the cell surface. Since β2GPI lacks intracellular domains we searched the possibility that anti-β2GPI antibodies recognize the CD40 molecule, which is capable to initiate signalling. The reason for this is that engagement of CD40 (by antibodies or CD40L) resembles many of the unexplained consequences of anti-β2GPI binding as (i) NFkB expression, (ii) induction of adhesion molecules (E-selectin, VCAM) and tissue factor on the surface of endothelial cells, (iii) secretion of proinflammatory cytokines and (iv) platelet activation.

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42B

SUCCESSIVE PREGNANCY OUTCOMES WITH LOW MOLECULAR WEIGHT HEPARIN AND ASA

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BACKGROUND: Many groups have reported low molecular weight (LMW) heparin ± aspirin (ASA) treatment for women with immunological or coagulation abnormalities and the success rates are given for index pregnancies in a study setting. In a routine clinical setting, women frequently have at least one pregnancy subsequent to study completion.

OBJECTIVE: To determine the live birth rate in women with a history of recurrent pregnancy loss (RPL) and at least 2 pregnancies treated with LMW heparin and ASA.

METHODS:. We prospectively followed women through all pregnancies subsequent to participation in a randomized clinical trial evaluating LMW heparin and ASA. Inclusion criteria for the first trial required that all women had a history of either primary or secondary RPL defined as ³ 2 serial losses in the absence of anatomic, hormonal, or genetic abnormalities. In addition, all women were positive for at least one of the following: anti-ds or ss DNA or anti-nuclear antibodies, anti-lymphocyte IgM, anti-cardiolipin IgG, or a lupus anticoagulant. All women received LMW heparin and ASA in each subsequent pregnancy and were included in this observational study regardless of their outcome in the initial study pregnancy.

RESULTS: Forty-four women had ³ 2 pregnancies treated with LMW heparin and ASA. There were 31 live births in the first pregnancy (70.5%), 10 spontaneous abortions (22.7%), and 3 still births (6.8%). In the second treated pregnancy, regardless of the outcome in first, there were 32/44 live births (77.4%): 8/13 (61.5%) live births in women after an initial failure and 24/31 (80.5%) live births after an initial success. Eleven of the 44 women had a third treated pregnancy, 3/44 had a fourth pregnancy, and 1 had a fifth treated pregnancy. Cumulatively, the number of women with at least one live birth after 2 pregnancies treated with LMW heparin and ASA was 39/44 (88.6%) and 42/44 had at least one live birth (95.5%) after ³ 2 treated pregnancies.

CONCLUSIONS: We observed a high success rate (95%) over a number of pregnancies with LMW heparin and ASA treatment for women with RPL. While randomized clinical trials may provide a snapshot of the efficacy of a therapy, they do not reflect the clinical reality of coping with and treating RPL throughout subsequent pregnancies. Whether the eventual pregnancy successes were a direct result of the LMW heparin and ASA, or whether the treatment was secondary or even inconsequential to the perseverance and determination of our patients (as evidenced by the successful pregnancy that occurred only after a fifth attempt in one woman), the overwhelming majority had at least one live birth on treatment.

1609

Effects of EPCR Gene Polymorphisms, Anti-EPCR Autoantibodies and SLE Disease Activity on sEPCR Levels: Multiple Variables May Impact Coagulation Risk in SLE. Tetsuya Horita, Kenaz Thomas, Ewa Olech, Stan Kamp, Eric Finley, Joan T. Merrill. Oklahoma Medical Research Foundation, Oklahoma City, OK

Purpose: The antiphospholipid syndrome (APS) is characterized by thrombosis and autoantibodies to coagulation proteins including several interacting members of the protein C anticoagulant pathway, including the endothelial protein C receptor (EPCR). The aim of the current study was to investigate, in a population with SLE, the relative impact of anti-EPCR autoantibodies, genetic polymorphisms of EPCR and SLE disease activity on soluble levels of EPCR (sEPCR), which have previously been described as elevated in SLE (Kurosawa Blood 1998).

Methods: 94 patients who met 1982 (rev) criteria for SLE were studied (29 African descent, 8 Native Am, 2 Asian, 54 Caucasian, of whom 12 were men and 82 women). 11/94 met Sapporo criteria for APS. DNA was genotyped for two common EPCR polymorphisms (SNPS) (rs867186 A/G, associated with high sEPCR, and rs9574 G/C, associated with high levels of activated anticoagulant protein C) using Li-Cor automated sequencers. Plasma sEPCR levels were measured by ELISA and a direct binding ELISA was developed and standardized against 67 healthy controls to detect anti-EPCR autoantibodies.

Results: Average sEPCR levels in this SLE population were 230 ng/ml confirming the previous report of elevated sEPCR in SLE. Gender and ethnicity had no effect on these levels. A trend was observed of increased mean sEPCR in patients with low BILAG scores (lowest quartile BILAG < or = 4) with 214 ng/ml vs highest quartile (BILAG > or = 8) 188 ng/ml. 11 patients with a minimum of an 8 point difference in BILAG scores at two different visits were also evaluated with mean sEPCR in lower BILAG scores 247ng/ml vs 223 with higher BILAG scores. Mean sEPCR for 16 patients with AG genotype for rs867186 was 293 ng/ml confirming some impact for this genotype. 13 of the 94 patients were positive for anti-EPCR autoantibodies of which 5 had elevated sEPCR (mean 491 ng/ml).

Conclusions: Our data are consistent with previous reports that sEPCR levels are increased in patients with SLE with or without the rs867186 genotype, and further suggest a possible impact of disease activity and autoantibodies to EPCR.

Disclosure: J.T. Merrill, None.

1610

Comparison of Clinical Events in Patients with Consistent and Fluctuating Lupus Anticoagulant Results. Christine A Clark, Karen A. Spitzer, Carl A. Laskin. University of Toronto and LifeQuest Centre for Reproductive Medicine, Toronto, ON, Canada

Purpose: The lupus anticoagulant (LAC) is frequently measured when patients present with clinical features of systemic lupus erythematosus (SLE), antiphospholipid syndrome (APS), or unexplained recurrent pregnancy loss (RPL; women with ≥ 2 losses, not fulfilling classification criteria for APS). Once found positive (and confirmed at least 6 weeks later), subsequent measurements are not often performed. We determined how many patients attending our lupus and/or RPL clinics had consistently positive LAC results over a 5-year period and whether the consistency of results has clinical correlations.

Methods: We performed a database search and chart review to determine LAC results and clinical events for patients who had ≥3 LAC tests over 5 years from 2000-2004. Demographic, diagnostic, obstetric, and thrombotic histories were obtained from charts. LAC was measured using a panel of tests including dilute PT, DRVVT, LA sensitive PTT, and KCT; a confirmed prolonged result for ≥1 of these tests constituted a positive result. LAC values were tabulated and patients assigned to 1 of 3 groups: consistently positive; consistently negative; and fluctuating. Prevalences were compared using z tests with 95% confidence intervals for the differences.

Results: Eighty patients had ≥ 3 LAC tests performed over 5 years: 47 patients were consistently negative, 19 were consistently positive, and 14 had fluctuating results over the 5 years. The frequencies of clinical events associated with each group are shown below.

Variable	% Neg LAC	% Pos/Neg LAC	% Pos LAC
SLE	53.2	57.1	10.5
APS	6.4	7.1	52.6
RPL	27.7	28.6	0
SLE/APS	0	0	36.8
Hx Thrombosis	10.6	0	52.6
Still Birth Ever	10.6	0	73.6
Live Birth Ever	60.0	85.7	21.1

Conclusions: Patients with consistently negative LAC results did not have significantly different frequencies of clinical events than patients with fluctuating results. However, both groups were significantly different from the group with consistently positive results. These observations suggest that LAC levels should be regularly monitored in young women with SLE and/or RPL who have an initially positive set of test results as the levels can fluctuate in about 40% of patients. Women with consistently positive results appear to have a much poorer obstetric prognosis.

Disclosure: C.A. Clark, None; K.A. Spitzer, None; C.A. Laskin, None.

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Incidence of Thrombotic Events in Systemic Lupus Erythematosus Patients with High Levels of Homocysteine and Positive Anti-Cardiolipin Antibodies. Ewa Olech, Paul Kamp, Ranit C Shriky, Stan Kamp, Kathleen O'Brien, Joan T Merrill. Oklahoma Medical Research Foundation, Oklahoma City, OK

Purpose: Hyperhomocysteinemia and anticardiolipin antibodies are associated with increased risk for thrombosis, which is a frequent complication of systemic lupus erythematosus (SLE). The current study aimed to determine if high homocysteine levels and anti-cardiolipin antibodies are associated with an increased number of thrombotic events in SLE patients.

Materials and Methods: 107 SLE patients from the Oklahoma Lupus Cohort were evaluated between March 2003 and April 2005. Plasma homocysteine levels and anti-cardiolipin antibodies were measured and the history of thrombotic events was collected, including fetal losses, venous and arterial thromboses. The data were analyzed according to normal (< 11.4 micromol/l) and high homocysteine levels; and presence or absence of anti-cardiolipin antibodies (aCL).

Results: There were 95 (88.8%) females and 12 (11.2%) males in the study group. Total number of the thrombotic events was 85 with a mean of 0.79 events per patient, suggesting a very high incidence in this population. Twenty five (23.4%) patients had elevated homocysteine levels (prevalence of hyperhomocysteinemia in general population: 5-7%). Out of those 25 patients, 12 had at least 1 thrombotic event. The mean number of thrombotic events in the hyperhomocysteinemic group was 1.4 vs only 0.61 in the group of patients with normal homocysteine.

50 (46.7%) patients had anti-cardiolipin antibodies. There were more thrombotic events in the aCL positive vs negative patients (0.94 vs 0.67), but the total number of patients with history of thrombosis and positive aCL was 21 vs. 22 patients with prior thrombosis and no known aPL. Although some with previous positive aCL might be missed, full record review and multiple testing has been performed on this population.

The group with positive anti-cardiolipin antibodies and elevated homocysteine levels had the highest mean number of thrombotic events (1.53 events per patient) vs aCL negative/ normal homocysteine group had the lowest (0.55).

Conclusions: Hyperhomocysteinemia is associated with thrombosis in SLE patients and might have an effect unrelated to antiphospholipid antibodies.

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Homocysteine Levels, Cardiovascular Risk Factors and Disease Duration are the Major Predictors of Coronary Calcification Measured by Electron Beam Computed Tomography in SLE Patients Compared to Age, Gender, and Race Matched Controls. Joan Von Feldt[1], E Nackos[1], L Scalzi[2], S Morthala[1], A Cucchiara[1], A Van Dyke[1], A Chander[1], E Gehrie[1]. [1]University of Pennsylvania, Philadelphia, PA;[2]University Hospitals Cleveland, Cleveland, OH

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Antiphospholipid Syndrome in Systemic Lupus Erythematosus: Is the Whole Greater Than the Sum of Its Parts?

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Systemic lupus erythematosus (SLE) is the prototypic, non–organ-specific autoimmune disease. When coexisting with the antiphospholipid syndrome (APS), the disease can be complicated by increased hypercoagulability that may exacerbate many of the more typical features. This article compares the manifestations of SLE in the presence and absence of antiphospholipid antibodies (aPLs), the hallmark autoantibodies of APS. The authors wish to determine if individuals who have SLE with aPL present a different clinical picture or appear to be at greater risk of developing certain features compared with those who do not have aPL.

Systemic lupus erythematosus and primary APS share several features but also have some unique distinguishing characteristics. Recurrent miscarriage and thrombosis form the clinical classification criteria for APS but not SLE (Box 1) [1–3]. For many years, before APS was recognized formally as a separate syndrome, rheumatologists would classify patients who have APS as having SLE while acknowledging that such individuals would be unlikely to manifest many of the signs and symptoms of classical SLE such as arthritis, photosensitivity, leukopenia, and glomerulonephritis. Despite obvious distinctions between the

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Box 1. Preliminary criteria for classification of antiphospholipid syndrome

Clinical criteria

Vascular thrombosis
Adverse pregnancy outcome

- At least one unexplained pregnancy loss beyond 10 weeks gestation
- Three or more unexplained, consecutive pregnancy losses before 10 weeks gestation
- One or more premature births of a normal neonate at or before 34 weeks' gestation because of pre-eclampsia or eclampsia, or placental insufficiency

Laboratory criteria

Moderate or high titer IgG or IgM anticardiolipin antibody measured on two or more occasions at least 6 weeks apart
Circulating anticoagulant measured on two or more occasions at least 6 weeks apart

Data from Wilson WA, Gharavi AE, Koike T, et al. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome. Arthritis Rheum 1999;42:1309–11.

two entities, it remains apparent that APS may, in many cases, be considered a subset of SLE.

Primary antiphospholipid syndrome

APS has been classified and established as a distinct clinical entity associated with thromboembolism, adverse pregnancy outcome, thrombocytopenia, Raynaud's phenomenon, and livedo reticularis in the presence of moderate or high levels of aPLs [1]. Other manifestations, most attributable to thrombotic events, have been described [4].

APLs are a family of antibodies directed against anionic, phospholipid proteins. In 1952, Conley and Hartmann first described a circulating anticoagulant associated with a hemorrhagic disorder in SLE [5]. This antibody came to be known as the lupus anticoagulant (LAC) and frequently is associated with a biologic false-positive test for syphilis (BFP). In 1963, Bowie et al [6] were the

first to report that patients who have an LAC actually experienced thrombotic events rather than hemorrhage. Recurrent pregnancy loss (RPL) was noted to be associated with LAC in the mid 1970s, and in the early 1980s, many reports of recurrent thrombosis and pregnancy loss in the context of LAC were published [7,8]. Finally in 1983, Harris et al established the association of the BFP, LAC, and anticardiolipin antibodies (aCL) [9]. Since that time, a family of aPL has been described with varying reports of clinical associations. In addition to the LAC and IgG and IgM aCL, anti-β2 glycoprotein I (β2GPI) and IgA aCL are included in this family. Antibodies to other negatively charged phospholipids such as phosphatidylserine, phosphatidylinositol, phosphatidylethanolamine, and phosphatidic acid appear to be of such limited clinical significance that, outside of a research environment, there is little reason to assay for their presence. Those antibodies demonstrating the most consistent association with clinical manifestations are the IgG and IgM aCL and the LAC.

Since the early work of Harris et al [9], several workshops have established classification criteria for APS, although inconsistent laboratory methodology for the detection of aCL antibodies and the LAC remains an issue [10,11]. The major manifestations of APS are thrombosis and adverse pregnancy outcome, especially RPL. Insights into the clinical manifestations of SLE in the presence of aPL will most likely be apparent with an analysis of vascular and pregnancy-related events.

Systemic lupus erythematosus and antiphospholipid antibodies

Anticardiolipin antibodies can be found in 2% to 5% of a normal population [11,12]. These antibodies increase in prevalence with increasing age, with IgG and IgM aCL being observed in 12% to 52% of an elderly population [13]. The frequency of the LAC is fairly well established in patients who have SLE. Approximately one-third of patients are positive [14]. In contrast, reports of aCL positivity in SLE vary considerably, from 23% to 47% [15–17]. Anti-β2GPI is found in 20% of lupus patients [17,18]. The question that arises from these data is whether aPL-positive lupus patients have an increase in thrombotic or adverse pregnancy events compared with those who do not have such antibodies. A second question, perhaps not as apparent, is whether certain vascular and pregnancy-related manifestations that have been ascribed to lupus, are seen mainly or exclusively in those who have aPL.

Classification of syndromes with antiphospholipid antibodies

The simplistic classification system used by most physicians is that of primary and secondary APS. The former is diagnosed in patients who have clinical features consistent with those defined in the preliminary classification criteria [1].

The latter is diagnosed in those who have a specific disorder, usually a connective tissue disease, who also have thrombotic events or adverse pregnancy outcomes in the presence of aPL. This classification system implies that there exists a clear distinction between APS and the connective tissue disease, but this is frequently not the case. Unfortunately, the most frequent scenario is also the most confusing: secondary APS in patients who have SLE.

Alarcon-Segovia proposed a modified classification that accepted the designations primary and secondary APS but added an intermediate group, which he named APS with lupoid features [19,20]. This group has features consistent with primary APS but fulfills fewer than four classification criteria for SLE (Fig. 1). Although this division into three subtypes of APS is not formally accepted, the concept of patients who might be experiencing a continuum of clinical manifestations helps to explain the clinical course observed in many individuals who have aPL and distinguishes primary APS from SLE and illustrates the relationship of APS as a subset of SLE in many other patients.

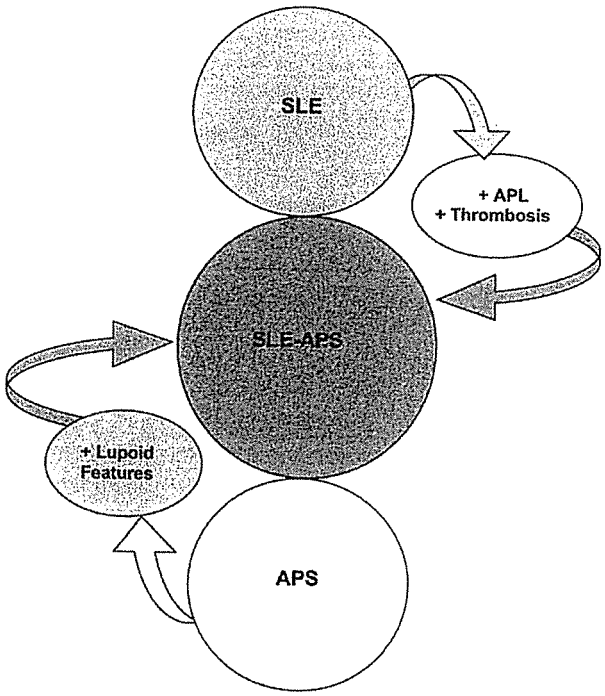

Fig. 1. The relationship of APS to SLE: APS can exist as a primary form, secondary to a connective tissue disease (usually SLE), or may be a primary form with some clinical features of SLE. The latter category can be referred to as an intermediate syndrome or APS with lupoid features.

Regardless of how these conditions are classified, one must distinguish those who have aPL from those who have aPL and clinical disease. In those who have secondary APS in association with SLE, clinical features that might be consistent with APS may not be caused by the presence of aPL. Indeed, these manifestations may be caused by some other aspect of lupus. For example, pregnancy loss may be caused by active renal disease, a flare of lupus, or even congenital heart block in the fetus (associated with anti-Ro/La antibody). Similarly, thrombosis may be caused by heavy proteinuria, which in turn leads to a hypercoagulable state independent of aPL. The question remains however, regarding the influence of the presence of aPL on SLE-specific disease manifestations and prognosis. It can be extremely difficult to tease apart clinical manifestations and attribute them to either APS or SLE when both conditions are present, and indeed, this distinction only becomes significant if therapeutic intervention differs depending upon disease attribution.

Hypercoagulability, antiphospholipid antibodies, and systemic lupus erythematosus

The first described and hallmark manifestation in individuals who have aPL is thrombosis, either venous or arterial, regardless of vessel size. This underlying pathogenesis accounts for the major manifestations including deep venous thrombosis (DVT), stroke or other ischemic cerebral event, and pregnancy loss. It is therefore reasonable to assume that aPL may adversely affect the prognosis of patients who have SLE. Several studies have shown that there is a strong association between the presence of aPL and thromboembolism in patients who have SLE. An extensive review by Love and Santoro [15] summarized data showing that thromboembolic events occurred in 53% of 160 patients who had a LAC and in 12% of 338 patients who did not have this antibody [6,21–28]. In those who were aCL positive, 40% of 300 had thrombotic events, compared with 18% of 364 patients who were antibody negative [21,22,29–34]. Therefore the presence of aPL in the setting of SLE is associated with an increased prevalence of thromboembolism compared with patients who did not have aPL.

There appears to be an interaction between the lupus disease process and aPL that causes an increase in thrombotic events in patients who have SLE and aPL. In patients positive for aPL associated with non-SLE conditions, the frequency of thrombotic events was lower (22% of 328 patients) than that in a group of aPL-positive patients who had SLE (42% of 340 patients) [27,35–42]. A more recent study of 1519 aPL-positive patients determined that cerebrovascular thrombosis was significantly more frequent in the aPL-SLE group compared with the primary APS group [43]. These investigators also showed that venous thrombosis occurred more frequently in those who had an LAC than in those who had aCL, whereas arterial thrombosis was more frequent in the aCL-positive group compared with those who had LAC. In a study comparing lung perfusion scintigraphy in patients who had SLE, SLE with APS (SLE–APS), and primary

APS, Aung et al found 43% of those who had SLE–APS to have segmental uptake defects compared with none in the other two groups [44]. Although the groups were small, the data suggest that those lupus patients who have aPL have a significantly higher risk of pulmonary thromboembolism compared with patients who have SLE alone. Similar observations have been made regarding the incidence of stroke in patients who have SLE–APS compared with SLE alone [45,46]. Regardless of the pathogenesis of hypercoagulability in the presence of aPL, there appears to be one or more factors specific to SLE that impact significantly upon the development of thromboembolism not seen in other conditions associated with aPL.

The prognosis of lupus appears to be impacted significantly by the presence of aPL. Because thrombotic events are more common in SLE–APS, it is not surprising that the mortality in lupus is increased when aPL are present. Ruiz-Irastorza et al found cumulative survival at 15 years to be lower in patients who had APS than in those who did not have APS (65% versus 90%, P=0.03) [47]. A retrospective analysis determined that 8-year survival in SLE patients who did not have APS was 98%. In those who had SLE–APS, it was 75%, and in those who had primary APS 83%. The presence of APS in SLE was associated significantly with higher mortality in this study (P=0.006) [48]. Regression analysis of the data showed that disease activity at onset, arterial thrombosis, thrombocytopenia, valvular heart disease, capillaritis, digital necrosis, and nephritis were independent mortality risk factors. This study illustrates how the interaction between manifestations of the underlying disease and those attributable to aPL combine to adversely affect longevity in this patient population.

Pregnancy in systemic lupus erythematosus and antiphospholipid syndrome

Insights into the clinical manifestations of SLE in the presence of aPL are most apparent upon analysis of vascular and pregnancy-related events, as adverse pregnancy outcome can be a significant problem in active SLE and APS. Indeed, pregnancy loss is one of the two clinical classification criteria of APS (see Box 1), and this issue is one that has attracted much attention among rheumatologists and obstetricians, attesting to its clinical importance.

Assessment of any woman who has a medical problem contemplating pregnancy requires an appreciation of both maternal and fetal issues. Therefore, one must address the effect of the pregnancy on the disease and the effect of the disease on the pregnancy. Ideally, a prepregnancy evaluation should be undertaken to appropriately plan the pregnancy enabling coordination of the efforts of both the rheumatologist and obstetrician. Unfortunately, unplanned pregnancies are almost the norm and require more urgent reactive rather than proactive evaluation and planning.

Pregnancy loss is the most controversial adverse outcome in both SLE and APS. The obvious questions are whether the problem in lupus is restricted

to those who have aPL and if the antibody exacerbates an already significant problem. APLs have been described in otherwise healthy women who have RPL, although with a frequency that is lower than might be expected. The LAC occurs in 15% of women who have RPL, while aCL might occur in 12% of a similar population [49–53]. Petri and Allbriton observed 13.1% pregnancy loss in a large lupus cohort [54]. This frequency appears to be representative when compared with other series of pregnant lupus patients [55–59]. In the presence of aPL, pregnancy loss appears to be more common in women who have SLE [60–63]. Between 50% and 74% of lupus patients who have a history of RPL have an LAC, and up to 36% have aCL [60,61].

In the presence of aPL, pregnancy loss can occur at any stage of pregnancy, although the classic description is that of late pregnancy loss. Both early and late pregnancy loss in lupus can be attributed to factors other than aPL, including active disease and an exacerbation of underlying renal disease. It is always problematic to assess the etiology of very early pregnancy loss, and even more difficult to determine the role of aPL in early pregnancy loss in lupus. The case becomes clearer if a patient who has SLE and aPL has a history of RPL even when her disease is under good control.

Later pregnancy losses may be assessed more readily for etiology. APLs have been associated with the development of placental thrombosis leading to placental insufficiency and intrauterine growth restriction (IUGR) culminating in fetal demise [63]. In the context of active lupus, the etiologic evidence for late pregnancy loss is far more subjective if placental pathology is either unavailable or inconclusive. The exception to this might be active renal disease with heavy proteinuria, which can precipitate pre-eclampsia. This in turn can lead to placental insufficiency with resultant IUGR, which occurs with increased frequency in lupus pregnancies with or without fetal demise. In this case, hypercoagulability is the common feature that could lead to a similar clinico–pathologic picture as APS. Therefore, although pregnancy loss may occur in SLE, the presence of aPL appears to place the pregnancy at greater risk. Unfortunately, data have yet to support intervention in pregnant women who have or do not have lupus when aPL is present if there is no previous history of pregnancy loss.

Pregnancy loss in SLE, regardless of aPL status, must be evaluated methodically. It would be wrong to make the a priori assumption that a loss is caused by either underlying disease or aPL. Any woman who has recurrent or late pregnancy loss should be investigated for anatomic, hormonal, or genetic abnormalities that might account for the losses, and it is essential that patients who have lupus be assessed using the same thorough protocol. Furthermore, if placental pathology is available and consistent with ischemic pregnancy loss even if aPL are negative, other thrombophilias such as protein C or S deficiency, factor V Leiden mutation, prothrombin gene mutation, antithrombin III deficiency, or hyperhomocysteinemia should be considered. The latter abnormality however, may be quite uncommon, because homocysteine levels are reduced by folic acid, a vitamin commonly taken by any woman contemplating a pregnancy.

Preterm birth (no later than 37 weeks' gestation) is common in SLE (40.5% of live births) [64,65] and can be related to disease activity, pre-eclampsia, placental insufficiency, or possibly premature rupture of the membranes [66,67]. In addition, preterm delivery also may be a reflection of advanced perinatal care where the obstetrician intervenes owing to threatened fetal demise [68]. Corticosteroids also have been associated with premature birth, but usually the neonate is appropriate size for gestational age [53]. Some authors also have suggested that the development of pre-eclampsia may be caused by the use of high doses of corticosteroids for disease control [69]. It is quite possible, however, that the steroids have little direct impact on the onset of pre-eclampsia and are actually an epiphenomenon in the etiology of pre-eclampsia. Rather, an exacerbation of disease activity or a flare of renal disease with heavy proteinuria may be the underlying problem, which is likely the reason for the institution of the high doses of corticosteroids. APLs have been associated with an increased frequency of pre-eclampsia, which could result in placental insufficiency, so the presence of aPL may be an additive factor in the high frequency of premature births commonly seen in women who have SLE.

Maternal complications are described in pregnant women with SLE. Although one would expect an increase in disease flares owing to the hyperestrogenic environment, the results of several studies are quite conflicting [64,66,70–77]. Among the medical complications are hypertension, diabetes mellitus, pre-eclampsia, and thrombosis [69,78]. Although these are more common in lupus, they may not be caused by disease activity. The higher risk of thrombosis is described in lupus pregnancies [78]. Even in uncomplicated pregnancies in healthy women, the third trimester, the immediate postpartum period, and after a cesarean section are characterized by hypercoagulability, and there is a greater risk associated with hypercoagulability in those who have SLE, which is increased further when aPL are present.

A particular problem arises when the woman who has SLE requires contraception. The easiest and most effective choice would be estrogen-containing oral contraceptive pills (OCP). Because lupus is a disease often exacerbated by a hyperestrogenic state, the use of OCP remains controversial owing to the risk of inducing a flare of the disease. If the patient is also positive for aPL, then OCP likely should be avoided. In this latter case, OCP may not only exacerbate the underlying disease (ie, SLE) but also place the woman at greater risk of thrombosis. Indeed, the presence of aPL should be considered at least a relative contraindication to the use of estrogen-containing OCP [79].

Women who have SLE or SLE–APS do not have any decrease in fertility. Although there is some debate regarding subfertility and an association with aPL, there is no good evidence to support that contention. There are however, two scenarios that may affect fertility adversely. The first is increased disease activity that could interfere with ovulation. The second issue is drug therapy. Cyclophosphamide may compromise ovarian function and potentially lead to ovarian failure.

It is possible for women who have SLE with or without APS to have infertility, as could any woman who does not have these conditions. Treatment for the

lupus patient may be problematic, however, owing to the hyperestrogenicity resulting from ovulation induction or superovulation therapy using exogenous follicle stimulating hormone (FSH). As with OCP, a hyperestrogenic environment may lead to exacerbation of SLE. There are cases of documented flares, but these have been mild [80]. Providing the disease is under good control, FSH therapy can be used with appropriate monitoring by the rheumatologist. The presence of aPL however, complicates the situation. Again, elevated estrogen levels may increase the tendency to thrombosis. Although there have been no large studies attesting to the safety of such therapy in women who have aPL, ovulation induction could be undertaken cautiously if there is no history of prior thrombosis, but the patient and all physicians involved should be counseled regarding potential risks.

Renal disease

The involvement of the kidney in SLE is known and described. Renal involvement has been part of the classification criteria of SLE from the outset [2,3]. With the exception of membranous disease, the renal lesions are inflammatory, targeting the glomerulus. Most patients who have SLE have some degree of renal disease, but it is not always clinically detectable without the benefit of immunofluorescence or electronmicroscopic evaluation of a kidney biopsy [81]. In the case of APS, renal involvement is not observed commonly. When present, the pathology is characterized by a thrombotic microangiopathy with thrombosis involving arterial and arteriolar lesions resulting in cortical ischemic atrophy [82,83]. The clinical manifestations of APS vascular nephropathy are characterized by hypertension, acute or chronic renal insufficiency, and low-grade proteinuria [84].

Daugas et al [84] retrospectively studied 114 patients who had SLE for renal disease in the presence or absence of aCL or LAC. The results revealed that APS nephropathy occurred in 32% of patients who had SLE in addition to lupus nephritis. The APS lesions were associated with LAC but not with aCL. Renal lesions occurred when there was evidence of extra-renal arterial thromboses and pregnancy loss but were not associated with venous thromboses. Most significantly, APS renal involvement appeared to be independent of the lupus-associated nephritis, and resulted in hypertension, renal insufficiency, and interstitial fibrosis. It is therefore likely that APS nephropathy contributes to increased morbidity and a poorer prognosis in this target lupus population because of its superimposition on lupus nephritis.

Renal transplantation in patients who have SLE appears to have a similar prognosis as transplantation in other conditions. The caveat to this reported observation, however, is the poorer prognosis seen in patients who have aPL [85–87]. Patients who have SLE–APS suffer an increased number of thromboembolic events posttransplant. There is a significant frequency of thrombotic

microangiopathy recurring in the graft and a higher incidence of thrombosis of the renal graft vein compared with lupus patients who do not have aPL.

Neurologic manifestations

Central nervous system (CNS) lupus is difficult to diagnose and manage. The manifestations have been variously reported as seizures, psychosis, stroke, transient ischemic attacks (TIA), chorea, cognitive dysfunction, and headaches. The pathology has been difficult to define, as it may be entirely normal. There is no gold standard test to diagnose CNS lupus, and physicians must rely on their clinical judgment. The most common neurologic manifestations of APS are stroke and TIA caused by arterial obstruction [88]. Although neuropsychiatric disorders have been linked to aPL, the association is tenuous. Neurologic involvement however, appears to be greater in those lupus patients who have aPL [15]. A review of the literature demonstrated neurologic disorders in 38% of LAC-positive patients compared with 21% in SLE patients lacking LAC. Similarly, 49% of lupus patients who had aCL had neurologic manifestations compared with 12% in aCL-negative lupus patients [15]. These data strongly suggest that CNS disease is more common in SLE–APS than in SLE without APS. Furthermore, as expected, aPL are associated more strongly with vascular neurologic events such as stroke, retinal artery occlusion, or amaurosis fugax. A recent multicenter study demonstrated a significant association of epilepsy with aPL positivity that appears to be secondary to CNS vascular disease [89].

Cardiac involvement

In 1924, Libman and Sacks described a verrucous endocarditis affecting the heart valves in patients who had SLE [90]. The lesions can be found in 30% to 50% of autopsy or echocardiographic studies but are not often of clinical significance during life. The valvular disease is usually mild and rarely leads to compromise of cardiac function. In APS, the valvular abnormalities are essentially identical to those seen in SLE. There are no data indicating that patients who have SLE–APS have a higher incidence of valvular disease compared with SLE alone, but Vianna et al [91] did find a higher incidence of valvular disease in patients who had SLE–APS compared with patients who had APS alone. These investigators did find, however, that 41% of SLE–APS patients who had valvular disease had significant mitral regurgitation, which was far in excess of that seen in SLE without aPL [92]. The more severe involvement in aPL-positive patients may be caused by a direct effect of aPL damaging the endocardium. Therefore, aPL appears to enhance the pathogenic mechanism operational in lupus as it affects cardiac valves. Moreover, there is an additional risk in the presence of both aPL and endocardial vegetations, which results in a higher frequency of embolic neurologic events [93,94].

Atherosclerosis in systemic lupus erythematosus and antiphospholipid syndrome

In 1976, Urowitz et al published a landmark paper documenting a bimodal mortality pattern in SLE [95]. Early deaths were caused by infection and renal disease, whereas later mortality was caused by atherosclerotic vascular disease. Recent studies observed that lupus patients have a 50-fold higher risk of developing cardiovascular disease and stroke beyond the conventional risk factors [96]. In the general population, aCL may be an independent risk factor for atherosclerotic vascular disease as evidenced by a 15% prevalence of aCL in middle-aged patients who had documented peripheral vascular disease [97]. In primary APS, IgG aCL was identified as an independent predictor of intima media thickness, a marker of atherosclerotic vascular disease [98]. Although lipid profile abnormalities have been described extensively in SLE, this is not the case in primary APS [99–101]. Alves and Ames contrasted the close association of dyslipidemia with atherosclerotic disease in SLE with the relative lack of such an abnormality in primary APS. They did, however, find a subgroup of patients who had primary APS lacking any lipid abnormality but having high titers of aCL, thereby implying a direct effect of aCL promoting atherosclerosis. Furthermore, these authors demonstrated an inhibitory effect of anti-β2GPI antibodies on the naturally occurring antioxidant paraoxonase [102]. Therefore in primary APS, atherosclerotic vascular disease appears to be promoted by aCL. Further evidence is provided that aPL may be an additive risk for atherosclerosis in SLE that is not accounted for by the traditional risk factors [103,104].

Other disease manifestations in systemic lupus erythematosus and antiphospholipid syndrome

Several other manifestations exist in SLE and APS that characterize each of these conditions. Thrombocytopenia is described in both disorders. It is frequently milder in APS than in SLE. There is no evidence to suggest that this cytopenia is more frequent in SLE–APS than in SLE alone. Similarly, Coombs-positive hemolytic anemia is described in APS and SLE. It is, however, more characteristic of lupus, and if occurring in primary APS, it may be an indicator of the evolution of APS into SLE.

The skin is a target in both conditions. Although livedo reticularis has been purported to be one of the hallmarks of APS, it appears to occur with equal frequency in SLE without aPL. Similarly, leg ulcers, digital gangrene, necrotizing purpura, and nailfold infarcts appear to be as common in SLE as in APS [105,106].

Catastrophic APS is a rare condition of small vessel vasculopathy affecting multiple organ systems associated with a high mortality [107]. It must be distinguished from thrombotic thrombocytopenic purpura and disseminated intravascular coagulation [108]. The distinguishing marker however, is aCL or

LAC positivity. This condition can occur in primary APS or SLE–APS where the presence of aPL renders patients who have SLE at risk for this often fatal complication.

Antiphospholipid syndrome and evolving systemic lupus erythematosus

The intermediate classification of APS with lupoid features proposed by Alarcon-Segovia [19] suggests that APS in some patients may be the first manifestation of SLE. Just as rheumatologists initially thought of APS as a subset of SLE, perhaps it may exist as evolving SLE. In a cohort of 165 patients who had APS followed over a median period of 78 months, Mujic et al [109] reported 3 of 80 patients who had primary APS developing features of SLE. Although one patient evolved into SLE after 4 years, the other two did not show features of lupus until 10 years after the diagnosis of primary APS. Similarly, a case report of a woman who had primary APS was diagnosed with SLE 15 years later [110]. In another case report of APS evolving into SLE 12 years after the first manifestations of APS, Derksen et al [111] suggest that a strongly positive antinuclear antibody in a patient who has primary APS may be the marker for later development of SLE. Carbone et al [112] reported that 3 (9%) of 33 patients who had a history of RPL and aPL developed features of SLE over a 6-year follow-up. The authors' group [113] found 3 of 32 women who had the same history of RPL also evolved into SLE or SLE–APS after a mean follow-up period of 12 years. Although some immunogenetic studies have been undertaken to determine if a particular haplotype is associated with APS occurring with SLE, the findings are rather preliminary. Despite the limited evidence, it is apparent that the evolution of APS into definite SLE does occur and may do so after many years.

Summary

SLE is the prototypical, autoimmune disease characterized by immune-mediated inflammation in multiple organ systems. Although many of the autoantibodies found in this condition appear to be merely markers of the presence of the disease or indicators of disease activity, some are definitely pathogenic. Morbidity and mortality in lupus are caused by the severity of the inflammation and the specific organ system involved. In addition, drug therapy often has significant impact on morbidity.

APS is an autoimmune disease characterized less by inflammation but more by hypercoagulability and thrombotic events. The disorder was initially described as a subset of SLE, but it is now known to exist as a separate entity in what is described as primary APS. Morbidity and mortality in this condition are caused by thrombosis and are dependent upon the organs affected. In addition, the impact of the disease on prognosis also may be determined by the type of

vascular involvement, with venous usually being of less concern than arterial. Therapy plays less of a role in morbidity than in SLE.

The combination of SLE and APS appears to be of greater concern than either entity alone. APS complicates SLE by adding a vaso-occlusive factor to the inflammatory component that adversely affects the prognosis of those who have lupus and aPL. The discovery of aPL in SLE and the diagnosis of the thrombotic complications associated with the APS aspect of the combined disorder, however, have significant implications therapeutically. Rather than instituting a regimen of corticosteroids and immunosuppressive agents with all of their attendant adverse effects, anticoagulation may be a safer and more appropriate therapeutic option. It is therefore incumbent upon the physician to clearly define the entity and evaluate the patient based upon a complete knowledge of the underlying disease processes.

References

[1] Wilson WA, Gharavi AE, Koike T, et al. International consensus statement on preliminary classification criteria for definite antiphospholipid syndrome. Arthritis Rheum 1999;42: 1309–11.

[2] Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271–7.

[3] Hochberg MC. Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1997;40:1725.

[4] Cervera R, Piette J-C, Font J, et al. Anti-phospholipid syndrome. Clinical and immunologic manifestations of disease expression in a cohort of 1000 patients. Arthritis Rheum 2002;46: 1019–27.

[5] Connley CL, Hartmann RC. Hemorrhagic disorder caused by circulating anticoagulant in patients with disseminated lupus erythematosus. J Clin Invest 1952;31:621–2.

[6] Bowie EJ, Thompson JH, Pascuzzi CA, et al. Thrombosis in systemic lupus erythematosus despite circulating anticoagulants. J Lab Clin Med 1963;62:416–30.

[7] Nilsson IM, Astedt B, Hedner V, Berezin D. Intrauterine death and circulating anticoagulant (anti-thromboplastin). Acta Med Scand 1975;197:153–9.

[8] Carreras LO, Defreyn G, Machin SJ, et al. Arterial thrombosis, intrauterine death and lupus anticoagulant: detection of immunoglobulin interfering with prostacyclin formation. Lancet 1981;1:244–6.

[9] Harris EN, Gharavi AE, Boey ML, et al. Anticardiolipin antibodies: detection by radioimmunoassay and association with thrombosis in systemic lupus erythematosus. Lancet 1983;2:1211–4.

[10] Lockshin MD, Sammaritano LS, Schwartzman SS. Validation of the Sapporo criteria for antiphospholipid antibody syndrome. Arthritis Rheum 2000;43:440–3.

[11] Fields RA, Toubbeh H, Searles RP, et al. The prevalence of anticardiolipin antibodies in a healthy elderly population and its association with antinuclear antibodies. J Rheumatol 1989; 16:623–5.

[12] Vila P, Hernandez MC, Lopez-Fernandez MF, et al. Prevalence, follow-up and clinical significance of the anticardiolipin antibodies in normal subjects. Thromb Haemost 1994;72: 209–13.

[13] Manoussakis MN, Tzioufas AG, Silis MP, et al. High prevalence of anticardiolipin and other autoantibodies in a healthy elderly population. Clin Exp Immunol 1987;69:557–65.

[14] McNeil HP, Chestemam CN, Krilis SA. Immunology and clinical importance of antiphospholipid antibodies. Adv Immunol 1991;49:193–280.

[15] Love PE, Santoro SA. Antiphospholipid antibodies: anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and non-SLE disorders. Ann Intern Med 1990; 112:682–98.
[16] Abu-Shakra M, Gladman D, Urowitz MB, et al. Anticardiolipin antibodies in systemic lupus erythematosus: clinical and laboratory correlations. Am J Med 1995;99:624–8.
[17] Sebastiani GD, Galeazzi M, Tincani A, et al. Anticardiolipin and anti-beta2GPI antibodies in a large series of European patients with systemic lupus erythematosus: prevalence and clinical associations. Scand J Rheumatol 1999;28:344–51.
[18] Bruce IN, Clark-Soloninka CA, Spitzer KA, et al. Prevalence of antibodies to B2-glycoprotein I in systemic lupus erythematosus and their association with antiphospholipid antibody syndrome criteria: a single centre study and literature review. J Rheumatol 2000;27:2833–7.
[19] Aracon-Segovia D, Cabral AR. The concept and classification of antiphospholipid/cofactor syndromes. Lupus 1996;5:364–7.
[20] Webe M, Hayem G, De Bandt M, et al. Classification of an intermediate group of patients with antiphospholipid syndrome and lupus-like disease: primary or secondary antiphospholipid syndrome? J Rheumatol 1999;26:2131–6.
[21] Derksen RH, Hasselaar P, Blizijl L, et al. Coagulation screen is more specific than the anticardiolipin antibody ELISA in defining a thrombotic subset of lupus patients. Ann Rheum Dis 1988;47:364–71.
[22] Petri M, Rheinschmidt M, Whiting-O'Keefe Q, et al. the frequency of lupus anticoagulant in systemic lupus erythematosus. A study of sixty consecutive patients by activated partial thromboplastin time, Russell viper venom time, and anticardiolipin antibody. Ann Intern Med 1987;106:523–31.
[23] Angles-Cano E, Sultan Y, Clauvel JP. Predisposing factors to thrombosis in systemic lupus erythematosus. J Lab Clin Med 1979;94:312–23.
[24] Boey ML, Colaco CB, Gharavi AE, et al. Thrombosis in systemic lupus erythematosus: striking association with the presence of circulating lupus anticoagulant. BMJ 1983;287:1021–3.
[25] Colaco CB, Elkon KB. The lupus anticoagulant. A disease marker in antinuclear antibody negative lupus that is cross-reactive with autoantibodies to double-stranded DNA. Arthritis Rheum 1985;28:67–74.
[26] Pauzner R, Rosner E, Many A. Circulating anticoagulant in systemic lupus erythematosus: clinical manifestations. Acta Haematol (Basel) 1986;76:90–4.
[27] Derksen RH, Bouma BN, Kater L. The prevalence and clinical associations of the lupus anticoagulant in systemic lupus erythematosus. Scand J Rheumatol 1987;16:185–92.
[28] Averbuch M, Koifman B, Levo Y. Lupus anticoagulant, thrombosis and thrombocytopenia in systemic lupus erythematosus. Am J Med Sci 1987;293:2–5.
[29] Tincani A, Meroni PL, Brucato A, et al. Anti-phospholipid and anti-mitochondrial type M5 antibodies in systemic lupus erythematosus. Clin Exp Rheumatol 1985;3:321–6.
[30] Meyer O, Piette JC, Bourgeois P, et al. Antiphospholipid antibodies: a disease marker in 25 patients with antinuclear antibody negative systemic lupus erythematosus (SLE). Comparison with a group of 91 patients with antinuclear antibody positive SLE. J Rheumatol 1987;14: 502–6.
[31] Fort JG, Cowchock FS, Abruzzo JL, et al. Anticardiolipin antibodies in patients with rheumatic diseases. Arthritis Rheum 1987;30:752–60.
[32] Manoussakis MN, Gharavi AE, Drosos AA, et al. Anticardiolipin antibodies in unselected autoimmune rheumatic disease patients. Clin Immunol Immunopathol 1987;44:297–307.
[33] Kalunian KC, Peter JB, Middlekauff HR, et al. Clinical significance of a single test for anti-cardiolipin antibodies in patients with systemic lupus erythematosus. Am J Med 1988;85: 602–8.
[34] Harris EN, Chan JK, Asherson RA, et al. Thrombosis, recurrent fetal loss, and thrombocytopenia. Predictive value of the anticardiolipin test. Arch Intern Med 1986;146:2153–6.
[35] Mannucci PM, Canciani MT, Mari D, et al. The varied sensitivity of partial thromboplastin and prothrombin time reagents in the demonstration of the lupus-like anticoagulant. Scand J Haematol 1979;22:423–34.

[36] Gastineau DA, Kazmier FJ, Nichols WL, et al. Lupus anticoagulant: an analysis of the clinical and laboratory features of 219 cases. Am J Hematol 1985;19:265–75.
[37] Jude B, Goudemand J, Dolle I, et al. Lupus anticoagulant: a clinical and laboratory study of 100 cases. Clin Lab Haematol 1988;10:41–51.
[38] Triplett DA, Brandt JT, Musgrave KA, et al. The relationship between lupus anticoagulants and antibodies to phospholipid. JAMA 1988;259:550–4.
[39] Mueh JR, Herbst KD, Rapaport SI. Thrombosis in patients with the lupus anticoagulant. Ann Intern Med 1980;92:156–9.
[40] Elias M, Eldor A. Thromboembolism in patients with the "lupus"-type circulating anticoagulant. Arch Intern Med 1984;144:510–5.
[41] Carreras LO, Vermylen JG. Lupus" anticoagulant and thrombosis–possible role of inhibition of prostacyclin formation. Thromb Haemost 1982;38:38–40.
[42] Waddell CC, Brown JA. The lupus anticoagulant in 14 male patients. JAMA 1982;248:2493–5.
[43] Soltesz P, Veres K, Lakos G, et al. Evaluation of clinical and laboratory features of antiphospholipid syndrome: a retrospective study of 637 patients. Lupus 2003;12:302–7.
[44] Aung W, Murata Y, Ishida R, et al. Comparison of lung perfusion scintigraphic findings in pulmonary thromboembolism in systemic lupus erythematosus, SLE plus antiphospholipid syndrome, and primary antiphospholipid syndrome. Nucl Med Commun 2000;21:299–304.
[45] Chaves CJ. Stroke in patients with systemic lupus erythematosus and antiphospholipid antibody syndrome. Curr Treat Options Cardiovasc Med 2004;6:223–9.
[46] Kitagawa Y, Gotoh F, Koto A, et al. Stroke in systemic lupus erythematosus. Stroke 1990;21: 1533–9.
[47] Ruiz-Irastorza G, Egurbide MV, Ugalde J, et al. High impact of antiphospholipid syndrome on irreversible organ damage and survival of patients with systemic lupus erythematosus. Arch Intern Med 2004;164:77–82.
[48] Reshetniak TM, Alekberova ZS, Kotel'nikova GP, et al. [Survival and prognostic factors of death risk in antiphospholipid syndrome: results of 8-year follow-up]. Ter Arkh 2003;75(5): 46–51 [in Russian].
[49] Edelman P, Rouquette AM, Verdy E, et al. Autoimmunity, fetal losses, lupus anticoagulant: beginning of systemic lupus erythematosus or new autoimmune entity with gynaeco-obstetrical expression? Hum Reprod 1986;1:295–7.
[50] Cowchock S, Smith JB, Gocial B. Antibodies to phospholipids and nuclear antigens in patients with repeated abortions. Am J Obstet Gynecol 1986;155:1002–10.
[51] Howard MA, Firkin BG, Healy DL, et al. Lupus anticoagulant in women with multiple spontaneous miscarriage. Am J Hematol 1987;26:175–8.
[52] Petri M, Golbus M, Anderson R, Whiting-O"Keefe Q, Corash L, Hellmann D. Antinuclear antibody, lupus anticoagulant, and anti-cardiolipin antibody in women with idiopathic habitual abortion. A controlled, prospective study of forty-four women. Arthritis Rheum 1987;30: 601–6.
[53] Laskin CA, Bombardier C, Hannah ME, et al. Prednisone and aspirin in women with autoantibodies and unexplained recurrent fetal loss. N Engl J Med 1997;337:148–53.
[54] Petri M, Allbritton J. Fetal outcome of lupus pregnancy: a retrospective case-control study of the Hopkins Lupus Cohort. J Rheumatol 1993;20:650–6.
[55] Engelbert HJ, Derue GM, Loizou S, et al. Pregnancy and lupus: prognostic indicators and response to treatment. Q J Med 1988;66:125–36.
[56] Hanly JG, Gladman DD, Rose TH, et al. Lupus pregnancy: a prospective study of placental changes. Arthritis Rheum 1988;31:358–66.
[57] Lockshin MD, Druzin ML, Goei S, et al. Antibody to cardiolipin as a predictor of fetal distress or death in pregnancy patients with systemic lupus erythematosus. N Engl J Med 1985;313: 152–6.
[58] Lockshin MD, Qamar T, Druzin ML, et al. Antibody to cardiolipin, lupus anticoagulant, and fetal death. J Rheumatol 1987;14:259–62.
[59] Koskela P, Vaarala O, Makitalo R, et al. Significance of false positive syphilis reactions

and anticardiolipin antibodies in a nationwide series of pregnant women. J Rheumatol 1988;15: 70–3.
[60] Derue GJ, Englert JH, Harris EN, et al. Fetal loss in systemic lupus: association with anticardiolipin antibodies. J Obstet Gynaecol 1985;5:207–9.
[61] Clauvel JP, Tchobroutsky C, Danon F, et al. Spontaneous recurrent fetal wastage and autoimmune abnormalities: a study of fourteen cases. Clin Immunol Immunopathol 1986;39: 523–30.
[62] Lockshin MD, Druzin ML, Qamar MA. Prednisone does not prevent recurrent fetal death in women with antiphospholipid antibody. Am J Obstet Gynecol 1989;169:439–43.
[63] Rahman P, Gladman DD, Urowitz MB. Clinical predictors of fetal outcome in systemic lupus erythematosus. J Rheumatol 1998;25:1526–30.
[64] Johns KR, Morand EF, Littlejohn GO. Pregnancy outcome in systemic lupus erythematosus (SLE): a review of 54 cases. Aust N Z J Med 1998;28:18–22.
[65] Kleinman D, Katz VL, Kuller JA. Perinatal outcomes in women with systemic lupus erythematosus. J Perinatol 1998;18:178–82.
[66] Aggarawal N, Sawhney H, Vasishta K, et al. Pregnancy in patients with systemic lupus erythematosus. Aust N Z J Obstet Gynaecol 1999;39:28–30.
[67] Johnson MJ, Petri M, Witter FR, et al. Evaluation of preterm delivery in a systemic lupus erythematosus pregnancy clinic. Obstet Gynecol 1995;86:396–9.
[68] Clark CA, Spitzer KA, Nadler JN, et al. Preterm deliveries in women with systemic lupus erythematosus. J Rheumatol 2003;30:2127–32.
[69] Petri M. Hopkins Lupus Pregnancy Center: 1987 to 1996. Rheum Dis Clin North Am 1997;23: 1–14.
[70] Lockshin MD, Reinitz E, Druzin ML, et al. Lupus pregnancy. I. Case-control prospective study demonstrating absence of lupus exacerbation during or after pregnancy. Am J Med 1984;77: 893–8.
[71] Meehan RT, Dorsey JK. Pregnancy among patients with systemic lupus erythematosus receiving immunosuppressive therapy. J Rheumatol 1987;14:252–8.
[72] Petri M, Howard D, Repke J. Frequency of lupus flare in pregnancy: the Hopkins Lupus pregnancy Center experience. Arthritis Rheum 1991;34:1538–45.
[73] Urowitz MB, Gladman DD, Farewell VT, et al. Lupus and pregnancy studies. Arthritis Rheum 1993;36:1392–7.
[74] Ruiz-Irastorza G, Lima F, Alves J, et al. Increased rate of lupus flare during pregnancy and the puerperium: a prospective study of 78 pregnancies. Br J Rheumatol 1996;35:133–8.
[75] Le Huong Î, Wechsler B, Vauthier-Brouzes D, et al. Outcome of planned pregnancies in systemic lupus erythematosus: a prospective study on 62 pregnancies. Br J Rheumatol 1997;36: 772–7.
[76] Carmona F, Font J, Cervera R, et al. Obstetrical outcome of pregnancy in patients with systemic lupus erythematosus. A study of 60 cases. Eur J Obstet Gynecol Reprod Biol 1999;83: 137–42.
[77] Georgiou PE, Politi EN, Katsimbrei P, et al. Outcome of lupus pregnancy: a controlled study. Rheumatology (Oxford) 2000;39:1014–9.
[78] Yasmeen S, Wilkins EE, Field NT, et al. Pregnancy outcomes in women with systemic lupus erythematosus. J Matern Fetal Med 2001;10:91–6.
[79] Lakasing L, Khamashta M. Contraceptive practices in women with systemic lupus erythematosus and/or antiphospholipid syndrome: what advice should we be giving? J Fam Plann Reprod Health Care 2001;27:7–12.
[80] Huong du LT, Wechsler B, Vauthier-Brouzes D, et al. Importance of planning ovulation induction therapy in systemic lupus erythematosus and antiphospholipid syndrome: a single center retrospective study of 21 cases and 114 cycles. Semin Arthritis Rheum 2002;32:174–88.
[81] Golbus J, McCune WJ. Lupus nephritis. Classification, prognosis, immunopathogenesis and treatment. Rheum Dis Clin North Am 1994;20:213–42.
[82] Nochy D, Daugas E, Droz D, et al. The intrarenal vascular lesions associated with primary antiphospholipid syndrome. J Am Soc Nephrol 1999;10:507–18.

[106] Naldi L, Locati F, Marchesi L, et al. Cutaneous manifestations associated with antiphospholipid antibodies in patients with suspected primary antiphospholipid syndrome: a case-control study. Ann Rheum Dis 1993;52:219–22.
[107] Asherson RA, Cervera R, Piette JC, et al. Catastrophic antiphospholipid syndrome. Clinical and laboratory features of 50 patients. Medicine (Baltimore) 1998;77:195–207.
[108] Asherson RA, Cervera R, de Groot PG, et al. Catastrophic Antiphospholipid Syndrome Registry Project Group. Catastrophic antiphospholipid syndrome: international consensus statement on classification criteria and treatment guidelines. Lupus 2003;12:530–4.
[109] Muljic F, Cuadrado MJ, Lloyd M, et al. Primary antiphospholipid syndrome evolving into systemic lupus erythematosus. J Rheumatol 1995;22:1589–92.
[110] Al Attia HM. Progression of primary APS (Hughes syndrome) into serological SLE: case report. Rheumatol Int 2001;20:79–80.
[111] Derksen RH, Gmelig-Meijling FH, de Groot PG. Primary antiphospholipid syndrome evolving into systemic lupus erythematosus. Lupus 1996;5:77–80.
[112] Carbone J, Orera M, Rodriguez-Mahou M, et al. Immunological abnormalities in primary APS evolving into SLE: 6 years follow-up in women with repeated pregnancy loss. Lupus 1999; 8:274–8.
[113] Clark CA, Spitzer KA, Goldberg AS, et al. A. Long-term follow-up of women with autoantibodies and recurrent pregnancy loss (RPL) [abstract]. Arthritis Rheum 2002;6(Suppl):S45.

Decrease in Pregnancy Loss Rates in Patients with Systemic Lupus Erythematosus Over a 40-Year Period

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ABSTRACT. ***Objective.*** To determine if there has been a statistically significant change in pregnancy loss and preterm delivery rates in patients with systemic lupus erythematosus (SLE).

Methods. We analyzed the pregnancy outcomes of our SLE patients over the past 3 years and reviewed the literature over the past 40 years. We extracted pregnancy loss and preterm delivery data from reports of postdiagnosis SLE pregnancies. Studies were grouped into 5-year periods and weighted according to sample size. Group means, calculated for each study period, were plotted using linear regression to determine significance, and compared with population norms for the same periods.

Results. The rate of loss in SLE pregnancies over the past 40 years decreased from a mean of 43% in 1960–1965 to 17% in 2000–2003 ($r^2 = 0.648$). This approximates the pregnancy loss rate in the general US population. Preterm deliveries were not uniformly reported and were rarely stratified into spontaneous or physician-initiated. Prior to 1980, it was not possible to derive group means for each time period. From 1980 to 2002, however, there was a trend toward a decrease in preterm births in SLE pregnancies, although they continue to be more frequent in SLE than in the general population.

Conclusion. Improvements in disease management and perinatal monitoring have resulted in a significant decrease in pregnancy loss in SLE over the last 40 years and a trend toward decreased preterm deliveries over the last 20 years in comparison to the general population. These advances highlight the importance of collaboration between rheumatologists and perinatologists. Given these data, the description of SLE-associated pregnancy could be revised to reflect a more positive prognosis for mother and fetus. (J Rheumatol 2005;32:1709–12)

Key Indexing Terms:
SYSTEMIC LUPUS ERYTHEMATOSUS PREGNANCY LOSS
PRETERM DELIVERY RATES

Systemic lupus erythematosus (SLE) continues to be described as a condition associated with poor pregnancy outcome despite advances in disease management and perinatal care. We wanted to determine if there has been a statistically significant change in pregnancy loss and preterm delivery rates in women with SLE over the last 40 years in comparison with the general population.

MATERIALS AND METHODS

Patient selection. We reviewed the pregnancy outcomes (pregnancy loss, live birth, and preterm delivery) of our patients with SLE over the last 3 years. Preterm deliveries were defined as those spontaneously occurring at less than 37 weeks' gestation, in contrast to those precipitated by the physician as the result of deteriorating maternal or fetal well being.

Literature review. In a literature review using the US National Library of Medicine PubMed database, we extracted pregnancy loss (including both early and late losses) and preterm delivery data (whether specified as spontaneous or not) from literature published over the last 40 years for women diagnosed with SLE. Values for the total number of pregnancies, fetal loss rates, and preterm delivery rates were elicited from each study.

For comparison, 5-year grouped US population values for pregnancy losses from 1960 to 2000 and preterm births (defined as deliveries at < 37 weeks' gestation) from 1980 to 2000 were calculated from data provided by National Vital Statistics Reports[1,2].

Analysis. Data were analyzed using Sigma Stat version 4.0 (SPSS, Chicago, IL, USA). Mean values were calculated for 5-year periods from 1960 to 2003, and values were plotted using linear regression analysis (with 95% confidence intervals, CI) to determine the significance of any trends over time.

RESULTS

Pregnancy outcome in our clinic. Of 83 pregnancies in women with SLE attending our clinic over the last 3 years, 73 (83%) resulted in a live birth. Of the 73 deliveries, 50 had spontaneous onset of labor, 17 of which (34%) occurred prior to 37 weeks' gestation.

Literature review and analysis. We identified more than 35 studies published since 1963 with pregnancy loss data available for patients diagnosed with SLE[3-37].

Studies were collated into 5-year periods as some years had no available SLE pregnancy data. Because the sample mean is sensitive to a few unusually large or small observa-

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tions, we used a trimmed sample mean. Studies with results that differed significantly from other studies in the same 5-year time period (< 25th or > 75th percentile) were not included in the analysis. Results were weighted accordingly by combining raw data from studies grouped into the 5-year periods starting with 1963.

Change in fetal loss rate in SLE pregnancies. The rate of loss in SLE pregnancies decreased over the past 40 years from a mean of more than 40% to a mean of 17%. Despite interclinic variation within time periods, overall there has been a significant trend toward increased live birth rates (Figure 1; $r^2 = 0.648$). In comparison, pregnancy loss rates in the US have remained relatively stable since 1960: in 2000, it was reported that the pregnancy loss rate was 16.1%[1] (Figure 1).

Change in preterm delivery rates in SLE pregnancies. The definition of preterm delivery varied from ≤ 36 weeks to ≤ 38 weeks among available SLE studies from 1980 to 2002, and was not generally reported in the earlier literature. There was seldom any delineation between spontaneous or induced onset of preterm labor. We therefore analyzed data from individual studies (in contrast to grouped data) and found a trend toward a decrease in preterm delivery rate (< 37 weeks' gestation) from 1980 to 2000 (37.3% to 32%, respectively), but this was neither consistent nor statistically significant ($r^2 = 0.418$; Figure 2). In contrast, there has been a slight but significant increase in the percentage of preterm births in the US over the same period (from 9.4% in 1981 to 12.1% in 2002; $r^2 = 0.997$)[2].

DISCUSSION

Our review was hampered by inconsistent reporting and variable definitions of events in the SLE literature. The term "fetal loss" comprised losses at all stages of pregnancy (from embryonic to stillbirth), and in many instances, the term "preterm delivery" (defined as < 36 to < 38 weeks' gestation) was not stratified as spontaneous or induced. In addition, statistical analysis had to account for sample sizes that varied from 11 to 108. Our results must be interpreted in the context of these difficulties. Nevertheless, it is apparent that there has been a significant decline in pregnancy loss rates in SLE over the last 40 years compared to the relatively stable rate in the general population over the same time period.

There are a number of factors contributing to the decline in pregnancy loss (and potentially preterm births) among women with SLE from both the maternal and the fetal standpoint. Disease management, particularly the identification and treatment of secondary antiphospholipid syndrome (APS), may be the most significant clinical advance in the last 2 decades, although there is still debate regarding the optimum therapeutic regimen for women with a history of thrombosis and/or recurrent pregnancy loss. There is also recognition that inactive disease in contrast to stable disease is an important consideration in reducing both maternal and fetal morbidity[37].

In addition, as timing of delivery is a highly critical aspect of antenatal management, measures including fetal biometry, amniotic fluid volume, heart rate patterns, arterial and venous Doppler, and biophysical variables are now inte-

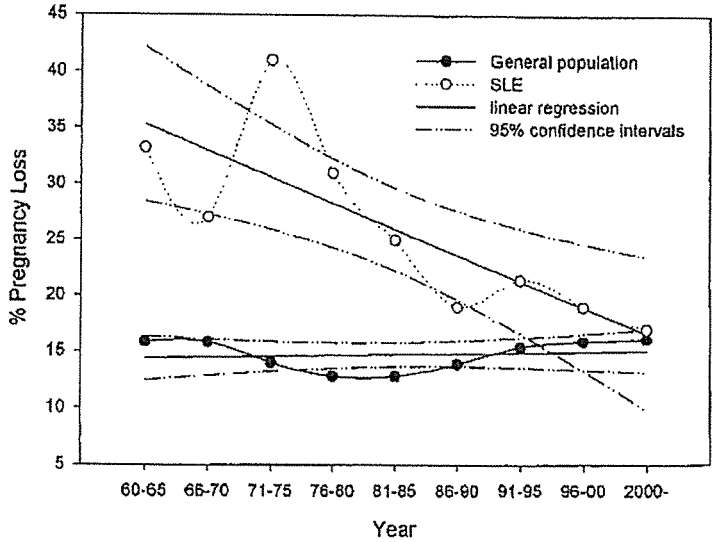

Figure 1. Change in rate of fetal loss in SLE pregnancies and in the US general population over that last 40 years. Data were grouped into 5-year periods (except the first period, 1963-65, and last period, 2001-2003).

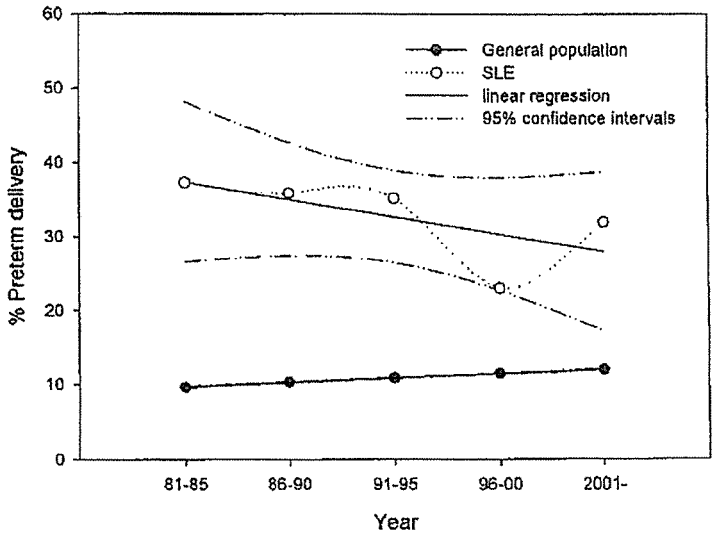

Figure 2. Change in rate of preterm deliveries (< 37 weeks' gestation, spontaneous and induced) in SLE pregnancies and in the US general population over the last 20 years.

gral parts of comprehensive fetal evaluation[38]. Clinicians are able to intervene and induce delivery in cases of deteriorating maternal or fetal health. Depending upon the gestational age, these induced deliveries might even be termed "fortuitous live births," as 20 years ago, no intervention would have been possible and the pregnancy would have terminated from either maternal or fetal causes with a resultant intrauterine death.

Kitridou and Goodwin[39] reviewed pregnancy outcomes in patients with SLE over the last half century and also reported rates approaching the population norm, but there was no mention of change in preterm deliveries. The increase in fetal survival due to improved pregnancy management was also raised by Petri and Allbritton[40], although the authors concluded (in 1993) that adverse pregnancy outcomes in lupus, including loss and preterm delivery, were still very common. In the intervening 12 years, that perception has gradually changed.

Pregnancy in women with SLE continues to be contraindicated in the context of severe organ disease or a history of life-threatening complications in previous pregnancies. For SLE patients without those superimposed conditions, however, there should be no contraindication for pregnancy, providing disease is clinically quiescent at the outset and there is appropriate planning and close prenatal monitoring.

We continue to advocate that SLE pregnancies be treated as high risk. However, based upon our review of the literature, we propose that the prevailing description of pregnancy in patients with SLE be revised to reflect advances in disease and perinatal management over the last 40 years.

REFERENCES

1. Ventura SJ, Mosher WD, Curtin SC, Abma JC, Henshaw S. Trends in pregnancies and pregnancy rates by outcome: estimates for the United States, 1976-96. National Center for Health Statistics. Washington, DC: DHHS Publication No. 2000-1934. Vital Health Stat 21 2000;56:1-47.
2. Martin JA, Hamilton BE, Sutton PD, Ventura SJ, Menacker F, Munson ML. Births: final data for 2002. Natl Vital Stat Rep 2003;52:1-113.
3. Mund A, Simson J, Rothfield N. Effect of pregnancy on the course of systemic lupus erythematosus. JAMA 1963;183:917-20.
4. Estes D, Larson DL. Systemic lupus erythematosus and pregnancy. Coin Obstet Gynecol 1965;8:307-21.
5. McGee CD, Makowski EL. Systemic lupus in pregnancy. Am J Obstet Gynecol 1970;107:1008-12.
6. Fraga A, Mintz G, Orozco J, et al. Sterility and fertility rates, fetal wastage and maternal morbidity in systemic lupus erythematosus. J Rheumatol 1974;1:293-8.
7. Grigor RR, Shervington PC, Hughes FRV, et al. Outcome of pregnancy in systemic lupus erythematosus. Proc Roy Soc Med 1977;70:99-100.
8. Zurier RB, Agyros TG, Urman JD, et al. Systemic lupus erythematosus: management during pregnancy. Obstet Gynecol 1978;51:178-80.
9. Devoe LD, Taylor RL. Systemic lupus erythematosus in pregnancy. Am J Obstet Gynecol 1979;135:473-9.
10. Tozman ECS, Urowitz MB, Gladman DD. Systemic lupus erythematosus and pregnancy. J Rheumatol 1980;7:624-32.
11. Hayslett JP, Lynn RI. Effect of pregnancy in patients with lupus nephropathy. Kidney Int 1980;18:207-20.
12. Houser MT, Fish AJ, Tagatz GE, et al. Pregnancy and systemic lupus erythematosus. Am J Obstet Gynecol 1980;138:409-13.
13. Zulman JI, Talal N, Hoffman GS, et al. Problems associated with the management of pregnancies in patients with systemic lupus erythematosus. J Rheumatol 1980;7:37-49.
14. Fine LG, Barnett EV, Danovitch GM, et al. Systemic lupus erythematosus in pregnancy. Ann Intern Med 1981;94:667-77.

15. Jungers P, Dougados M, Pelisser C, et al. Lupus nephropathy and pregnancy; report of 104 cases in 36 patients. Arch Intern Med 1982;142:771-6.
16. Varner MW, Meehan RT, Syrop CH, et al. Pregnancy in patients with systemic lupus erythematosus. Am J Obstet Gynecol 1983;145:1025-40.
17. Imbasciati E, Surian M, Bottino S, et al. Lupus nephropathy and pregnancy: a study of 26 pregnancies in patients with systemic lupus erythematosus and nephritis. Nephron 1984;36:46-51.
18. Lockshin MD, Reinitz E, Ruzin ML, et al. Lupus pregnancy. I. Case-control prospective study demonstrating absence of lupus exacerbation during or after pregnancy. Am J Med 1984;77:893-8.
19. Gimovsky ML, Montoro M, Paul RH. Pregnancy outcome in women with systemic lupus erythematosus. Obstet Gynecol 1984;63:686-92.
20. Mintz G, Niz J, Gutierrez C, et al. Prospective study of pregnancy in systemic lupus erythematosus. J Rheumatol 1986;13:732-9.
21. Borbrie G, Liote F, Houllier P, et al. Pregnancy in lupus nephritis and related disorders. Am J Kidney Dis 1987;9:339-43.
22. Meehan RT, Dorsey JK. Pregnancy among patients with systemic lupus erythematosus receiving immunosuppressive therapy. J Rheumatol 1987;14:252-8.
23. Simaopoulou-Mavridou A, Manoussakis MN, Maviridis AK, et al. Outcome of pregnancy in patients with autoimmune rheumatic disease before the disease onset. Ann Rheum Dis 1988;47:892-7.
24. McHugh NJ, Reilly PA, MacHugh LA. Pregnancy outcome and autoantibodies in connective tissue disease. J Rheumatol 1989;16:42-6.
25. Lockshin MD. Pregnancy does not cause systemic lupus erythematosus to worsen. Arthritis Rheum 1989;32:665-70.
26. Nossent JC, Swaak AJG. Systemic lupus erythematosus. IV. Analysis of the interrelationship with pregnancy. J Rheumatol 1990;17:771-6.
27. Wong KL, Chan FY, Lee CP. Outcome of pregnancy in patients with systemic lupus erythematosus: a prospective study. Arch Intern Med 1991;151:269-73.
28. Le Thi Huong D, Wechsler B, Piette J-C, Bletry O, Godeau P. Pregnancy and its outcome in systemic lupus erythematosus. QJM 1994;87:721-9.
29. Lima F, Buchanan NMM, Khamashta MA, Kerslake S, Hughes GRV. Obstetric outcome in systemic lupus erythematosus. Semin Arthritis Rheum 1995;25:184-92.
30. Johnson MJ, Petri M, Witter FR, Repke JT. Evaluation of preterm delivery in a systemic lupus erythematosus pregnancy clinic. Obstet Gynecol 1995;86:396-9.
31. Le Thi Huong D, Wechsler B, Vauthier-Brouzes D, et al. Outcome of planned pregnancies in systemic lupus erythematosus: a prospective study on 62 pregnancies. Br J Rheumatol 1997;36:772-7.
32. Rahman P, Gladman DD, Urowitz MB. Clinical predictors of fetal outcome in systemic lupus erythematosus. J Rheumatol 1998;25:1526-30.
33. Carmona F, Font J, Cervera R, Munoz F, Cararach V, Balasch J. Obstetrical outcome of pregnancy in patients with systemic lupus erythematosus. A study of 60 cases. Eur J Obstet Gynecol Reprod Biol 1999;83:137-42.
34. Kobayashi N, Yamada H, Kisheda T, et al. Hypocomplementemia correlates with intrauterine growth retardation in systemic lupus erythematosus. Am J Reprod Immunol 1999;42:153-9.
35. Georgious PE, Polii EN, Katsimbri P, Sakka V, Drosos AA. Outcome of lupus pregnancy: a controlled study. Rheumatology Oxford 2002;39:1014-9.
36. Cortes-Hernandez J, Ordi-Ros J, Paredes F, Casellas M, Castillo F, Vilardell-Tarres M. Clinical predictors of fetal and maternal outcome in systemic lupus erythematosus: a prospective study of 103 pregnancies. Rheumatology Oxford 2002;41:643-50.
37. Clark CA, Spitzer KA, Nadler JN, Laskin CA. Preterm deliveries in women with systemic lupus erythematosus. J Rheumatol 2003;30:2127-32.
38. Baschat AA, Hecher K. Fetal growth restriction due to placental disease. Semin Perinatol 2004;28:67-80.
39. Kitridou RC, Goodwin TM. The fetus in systemic lupus erythematosus. In: Wallace DJ, Hahn BH, editors. Dubois' lupus erythematosus. Philadelphia: Lippincott Williams & Wilkins; 2002:1023-40.
40. Petri M, Allbritton J. Fetal outcome of lupus pregnancy: a retrospective case-control study of the Hopkins Lupus Cohort. J Rheumatol 1993;20:650-6.

Incidence of Postpartum Thrombosis and Preterm Delivery in Women with Antiphospholipid Antibodies and Recurrent Pregnancy Loss

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ABSTRACT. ***Objective.*** To determine the frequency of preterm deliveries and postpartum thrombotic events (TE) in pregnancies resulting in live birth in women with antiphospholipid antibodies (aPL) and a history of recurrent pregnancy loss (RPL) but without prior TE.

Methods. We reviewed the pregnancy outcomes of women referred to our clinic with a history of RPL. Prepregnancy investigation of RPL included history of TE and aPL positivity (anticardiolipin IgG and lupus anticoagulant). We recorded use of anticoagulation therapy during and after pregnancy, obstetric outcome, gestational age at delivery, and postpartum course. Included in our study were women with unexplained RPL with no history of TE attending our clinic who subsequently had pregnancies that resulted in a live birth.

Results. Over a 5-year period, 260 women with RPL and no history of TE had a live birth at our clinic. Eighty-seven (33.5%) were positive for aPL and 173 (66.5%) were negative for aPL. Twenty-four percent of deliveries in the aPL-positive group occurred before 37 weeks' gestation compared to 9.8% of deliveries in the aPL-negative group ($p = 0.004$; 95% CI 0.052–0.234). There were no antepartum TE in either group. One woman in the aPL-positive group (1.1%) had a deep vein thrombosis 3.5 weeks postpartum while receiving prophylactic anticoagulant therapy, compared to none in the aPL-negative group.

Conclusion. A significantly higher proportion of aPL-positive patients had preterm deliveries compared to aPL-negative patients, but pregnancy-related TE was infrequent: 99.0% of aPL-positive women with a history of RPL and no prior TE who had a live birth at our clinic had an uneventful pregnancy, delivery, and postpartum course. (J Rheumatol First Release April 1 2007)

Key Indexing Terms:
PRETERM DELIVERY POSTPARTUM THROMBOSIS ANTIPHOSPHOLIPID ANTIBODIES
ANTICOAGULATION ANTICARDIOLIPIN LUPUS ANTICOAGULANT

Women with antiphospholipid antibodies (aPL) are considered at increased risk for recurrent pregnancy loss (RPL), intrauterine growth restriction, stillbirth, prematurity, and thrombotic events (TE)[1-3]. Despite continuing debate regarding appropriate therapy due to a dearth of well designed, appropriately powered studies[4,5], the standard of care for women with aPL and a history of RPL but not TE continues to include prophylactic anticoagulant therapy during pregnancy[5-7].

Studies investigating treatment regimens for aPL-positive pregnancy usually identify live birth rate as the primary outcome and the incidence of intrauterine growth restriction, placental infarction, prematurity, and preeclampsia as secondary outcomes. There is only limited literature available about the incidence of postpartum TE in this population[8-10].

The TERM Programme (Treatment and Evaluation of Recurrent Miscarriage), established in 1996, investigates about 300 new patients per year with a history of RPL. Those identified with hormonal, genetic, or anatomic abnormalities that might account for reproductive failures are referred for appropriate treatment or counseling. Those with immunologic and coagulation abnormalities are prospectively followed at our clinic from the prepregnancy to postpartum period.

We initiated this investigation because the necessity for postpartum anticoagulation in aPL-positive pregnancies (in the absence of prior TE) has not been determined and yet this regimen is becoming entrenched. We report our experience

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over a 5-year period comparing outcomes of aPL-positive and negative women without a history of TE whose pregnancies resulted in a live birth. We wanted to observe the frequency of pregnancy-related thrombosis in women with aPL but without a history of TE compared to women with a similar obstetric history, but no aPL, and also to evaluate the association between aPL positivity and preterm delivery.

MATERIALS AND METHODS

Study design. Patients with a history of RPL referred to our clinic over a 5-year period were prospectively followed throughout their pregnancies. We subsequently performed a chart review and collected demographic, clinical, and obstetric outcome data on patients whose pregnancies had progressed to at least 27 weeks. We restricted our study in this manner because we wanted to confine our analysis to pregnancies with a significant likelihood of live birth. Further, women in early pregnancy are less likely to experience any pregnancy-related TE[11]. We recorded anticoagulation use throughout pregnancy and the postpartum period.

Patients with significant comorbidity, including systemic lupus erythematosus and diabetes, were excluded from the study.

Definition of RPL. Patients were classified with RPL defined as at least 2 consecutive losses in the absence of anatomic (assessed by hysterosalpingogram or sonohysterogram), genetic (karyotype analysis of both partners), or hormonal (luteal-phase biopsy or mid-luteal-phase progesterone level) abnormalities. Early losses were defined as those occurring at < 14 weeks' gestation, late losses as those at ≥ 14 weeks' gestation.

Laboratory evaluation. Prenatal serum and plasma specimens were collected from each patient and stored at –80°C as part of routine investigation. Those with a positive aPL result were recalled to the clinic a minimum of 6 weeks later for collection of a second sample for confirmation purposes. The lupus anticoagulant (LAC) was measured using a panel of tests to optimize detection as described[12], including Russell's viper venom time (DRVVT), dilute prothrombin time, a lupus-sensitive partial thromboplastin time, and the kaolin-cephalin clotting time. Factor deficiencies were ruled out by repeat testing of prolonged results with 1:1 and 4:1 mixing with normal plasma. IgG anticardiolipin (aCL) levels were measured using INOVA Quantalite kits (Intermedico, Mississauga, ON, Canada). Results were derived from a standard curve, and values > 15 GPL units were considered positive based upon the mean + 2 standard deviation of the results of 162 normal sera. We did not include aCL IgM in our study because not all women were tested for that isotype.

aPL-positive group. This group comprised patients with a history of RPL who were positive for aCL IgG and/or LAC on at least 2 occasions, 6 weeks apart, but negative for anatomic, hormonal, or genetic investigations, and an index pregnancy that progressed to at least 27 weeks' gestation.

Comparator group. This group included aPL-negative women with a history of RPL with no anatomic, hormonal, or genetic abnormalities, and no history of TE, with at least one documented pregnancy (subsequent to the last spontaneous loss) that progressed to at least 27 weeks' gestation.

Statistical analysis. We used SigmaStat Version 3.0 software (SPSS Inc., Chicago, IL, USA) for statistical analysis. Proportions were compared using the z test (for population prevalence statistics) with Yates' correction factor. A p value < 0.05 was considered significant and 95% confidence intervals (CI) were calculated for differences and odds ratios. Where appropriate, Fisher's exact test was used for comparisons of proportions with expected cell values of less than 5.

RESULTS

Patients. The 2 comparator groups included 87 aPL-positive and 173 aPL-negative women; all had a history of RPL as defined above and none had a history of TE. There was no difference in the obstetric histories of women in each group (Table 1) with the following exception: significantly more women in the aPL-negative group had a history of at least one live birth compared to the aPL-positive group (72.8% vs 41.4%; p > 0.001, 95% CI 0.189–0.439), indicating that more women in the aPL-positive group had primary rather than secondary RPL. There was no difference in the proportion of each group with 2 or > 2 pregnancy losses (Table 1). There was no difference in the mean ages of the women in each group (32.0 vs 33.3 yrs; p = not significant).

Distribution of aPL. In the aPL-positive group, 54.0% of women were positive for LAC only; 28.7% were positive for aCL only; 17.2% were positive for both LAC and aCL. The LAC-specific partial thromboplastin time (PTT-LAC) was the most frequently seen LAC measured (74.2% of women with an LAC had a prolonged PTT-LAC), followed by the DRVVT (40.9%; Table 2).

Table 1. Obstetric histories of women with a history of recurrent pregnancy loss but not thrombotic events, with and without antiphospholipid antibodies (aPL). There was no difference between the 2 groups with the exception of live births: more aPL-negative women had at least 1 live birth prior to the index pregnancy.

Obstetric History	aPL-Positive, n = 87	aPL-Negative, n = 173	p
Pregnancies, mean ± SD, median (range)	3.5 ± 1.4, 3 (2–8)	3.9 ± 1.3, 4 (2–8)	NS
2 Early losses (%)	34 (39.1)	52 (31.0)	NS
≥ 3 Early losses (%)	45 (51.7)	105 (62.5)	NS
Early and late losses (%)	9 (10.3)	10 (6.0)	NS
Late losses only (%)	2 (2.3)	1 (0.6)	NS
Live birth ever (%)	36 (41.4)	126 (72.8)	< 0.001*

NS: not significantly different. * Power with α = 0.05: 0.998; 95% CI of the difference 0.189–0.439.

Table 2. Frequency and distribution of anticardiolipin (aCL) titers and specific lupus anticoagulant (LAC) tests in the aPL-positive group (n = 87). 15 women in the group were positive for both aCL and LAC. Nine patients in the LAC positive group (13.6%) had prolonged results for all 4 LAC tests.

Phospholipid Antibody	n Positive (%)
aCL IgG (all titers)	48/87 (55.2)
Frequency of titer ranges among aCL-positive results (n = 48)	
Low (15–25 GPL)	28 (58.3)
Moderate (26–50 GPL)	10 (20.8)
High (> 51 GPL)	10 (20.8)
LAC (≥ 1 test in panel)	66/87 (75.9)
Frequency of specific tests among LAC-positive results* (n = 66)	
Dilute PT	15 (22.7)
PTT-LAC	49 (74.2)
DRVVT	27 (40.9)
KCT	15 (22.7)

* Percentages do not sum to 100 because many patients were positive for more than one LAC test. Dilute PT: dilute prothrombin time; PTT-LAC: lupus anticoagulant-specific partial thromboplastin time; DRVVT: dilute Russell's viper venom time; KCT: kaolin-cephalin clotting time; GPL: IgG anticardiolipin units (negative: ≤ 15).

Women fulfilling criteria for the antiphospholipid syndrome (APS). Forty-five women in the aPL-positive group had ≥ 3 early losses and 11 had at least one late loss. Of those 56 women, 45 also had repeated moderate to high levels of aCL IgG and/or prolonged LAC. This latter group thereby satisfied obstetric and laboratory classification criteria for APS.

Anticoagulation therapy during and/or after pregnancy. Prenatal and/or postpartum anticoagulation therapy was given at the discretion of the attending physician. Prophylactic anticoagulation therapy was given during pregnancy to 71/87 aPL-positive patients: 44 received prophylactic doses of low molecular weight heparin (LMWH; dalteparin at 5000 IU once daily or a weight-adjusted equivalent) with low-dose aspirin (ASA, 81 mg/day); ASA only at a dose of 81 mg/day was taken by 27 women; 16 women received no treatment. Only 4 women in the aPL-positive group and none in the aPL-negative group received anticoagulation postpartum. ASA was discontinued at 35 weeks' gestation and not given postpartum, according to clinic policy.

Pregnancy-related TE. There were no episodes of thrombosis during any of the 260 pregnancies observed. There were no postpartum TE in the aPL-negative group. One (1.1%) woman in the aPL-positive group had a deep vein thrombosis 3.5 weeks postpartum while receiving prophylactic LMWH. She had a history of 2 early losses and was positive for high levels of both aCL IgG and LAC (PTT-LAC, dilute prothrombin time, and DRVVT). At 28 weeks' gestation, she developed high blood pressure, decreased platelets, and proteinuria, and was diagnosed with HELLP syndrome (hemolysis, elevated liver enzymes, and low platelets). Due to her deteriorating condition, a nonelective cesarean section was performed at 31 weeks' gestation. She developed hypertensive retinopathy postpartum; she had been receiving 5000 IU/day fractionated heparin throughout pregnancy and after delivery. This treatment was to continue until 6 weeks' postpartum. She was discharged from hospital 10 days after delivery and one week later, she reported leg pain. A deep vein thrombosis was confirmed by venogram 3 weeks' postpartum.

Preterm deliveries. Twenty-four percent of deliveries in the aPL-positive group occurred before 37 weeks' gestation compared to 9.8% of deliveries in the aPL-negative group ($p = 0.004$; 95% CI 0.052–0.234; Figure 1). There was no difference in the frequency of preterm deliveries in the aPL group between women with a history of 2 compared to > 2 prior pregnancy losses (30.4% vs 30.7%, respectively).

Within the 3 treatment groups in the aPL-positive group, preterm and term deliveries were differentially distributed (Table 2): in the group receiving ASA, 13/27 (48.1%) had preterm deliveries compared to 7/44 (15.9%) of women receiving LMWH/ASA ($p = 0.003$, power with $\alpha = 0.05$, 0.843; 95% CI for the difference 0.141–0.579) and 1/15 (6.3%) receiving no treatment ($p = 0.012$, power with $\alpha = 0.05$, 0.758; 95% CI for the difference 0.129–0.709). There was no difference in the frequency of term deliveries among untreated aPL-positive and aPL-negative women (93.8% vs 91.4%, respectively).

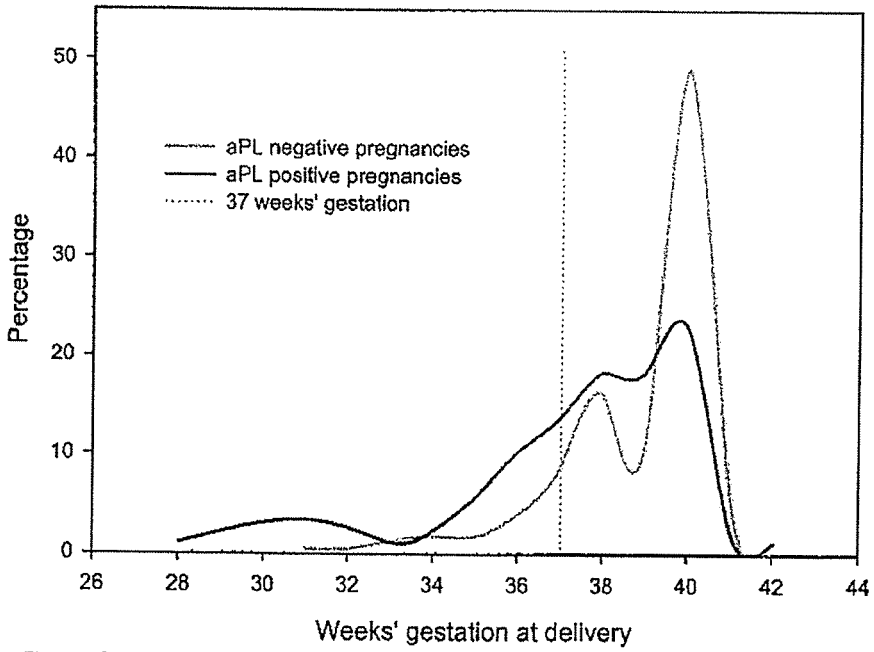

Figure 1. Comparison of preterm deliveries in aPL-positive versus aPL-negative pregnancies. There were significantly more preterm deliveries in the aPL-positive group (24.1% vs 9.8%; $p = 0.004$).

DISCUSSION

Management of TE in women with aPL outside the context of pregnancy is well described[13-15]. Several investigators have attempted to establish guidelines for the treatment of pregnant women with confirmed previous or current thromboembolism regardless of aPL status[16]. As Empson, *et al* and Derksen, *et al* have shown[4-6], there is also a large if disparate body of literature regarding management of pregnancy in women with aPL, although the primary outcome measure of experimental treatment protocols for this population is usually live birth rate and not reduction in TE during pregnancy or postpartum[5,7,17,18]. Despite the lack of evidence-based treatment guidelines[16] particularly for women with aPL in the postpartum period, anticoagulation during and after pregnancy is frequently described as the standard of practice[19-21]. However, as we have reported, management of patients with RPL with or without aPL and with or without a history of TE varies considerably from specialty to specialty[22], and cannot yet be considered either standardized or evidence-based.

In our RPL clinic over 5 years, we observed 260 pregnancies that resulted in a live birth in women with a history of at least 2 prior losses. One woman with aPL in the context of RPL with no prior thrombotic history had a postpartum TE compared to none in the group without aPL. An association between aPL positivity and postpartum TE in this population of women was not found. Postnatal anticoagulation did not eliminate the occurrence of a postpartum TE in our patient. Others have also found that TE can occur despite thromboprophylaxis during pregnancy in women with a history of prior events[23,24]. Of interest, in a prospective study of 125 pregnant women with a history of TE in the absence of known aPL or other prothrombotic conditions, Brill-Edwards, *et al*[25] found that antepartum thromboprophylaxis was unwarranted as the risk of recurrent thromboembolism was so low.

Although there was no difference in the number of prior spontaneous abortions between our 2 groups, more than 60% of our aPL-negative group with RPL had a history of at least one live birth compared to 40% of our aPL-positive group, suggesting an association between primary RPL and aPL positivity. We were also able to confirm an association between aPL positivity and prematurity, as there was a significant increase in preterm deliveries in our aPL-positive group compared to the aPL-negative group. This has been noted by ourselves and others regarding aPL in the context of systemic lupus erythematosus[26].

Table 3. Distribution of preterm deliveries among treatment groups in our aPL-positive population of women (n = 87) with a history of RPL. Results are expressed as percentages in each treatment group with either term or preterm deliveries. In the aPL-negative group, 17 (9.8%) women had preterm deliveries.

Treatment During Pregnancy	Term Deliveries, n (%)	Preterm Deliveries, n (%)
LMWH + ASA	37 (84.1)	7 (15.9)
ASA only	14 (51.9)	13 (48.1)
No treatment	15 (93.8)	1 (6.3)
All treatment groups	66 (75.9)	21 (24.1)

LMWH: low molecular weight heparin.

We noted a modest treatment effect among our patients that has not been previously reported. Of 16 aPL-positive patients who received no anticoagulation during pregnancy at the discretion of their physicians, 15 (93.8%) had term deliveries. A similar if slightly lower percentage (85%) receiving LMWH/ASA also had term deliveries. In contrast, only 14 of 27 (51.9%) women receiving only ASA had term deliveries. These sample sizes are small but the difference between the ASA and no-treatment groups was statistically significant. However, this observation should be considered with caution: this was not a randomized clinical trial comparing treatment effects. The differences we observed in preterm delivery rates may simply have been an epiphenomenon, and our observation is not in agreement with a metaanalysis of ASA consumption during pregnancy in which Kozer, *et al* concluded that ASA seemed to have a small but significant effect on reducing the rate of preterm deliveries in moderate to high-risk pregnancies[27].

Our study has limitations that should be noted. Our population was highly selected and thus our results are probably not widely generalizable. Second, although our population was large in comparison to many studies, it was still too small to establish an association or lack thereof between aPL and postpartum thrombosis. Finally, pre- and postpartum thromboprophylaxis was not protocolized, thus limiting our ability to comment on the efficacy of individual thromboprophylaxis regimens.

Defining RPL as ≥ 2 rather than the more commonly accepted ≥ 3 consecutive pregnancy losses may also be considered a weakness of our study by some. While rigid inclusion and exclusion criteria are required for randomized clinical trials evaluating therapeutic regimens, in our routine clinical practice with this population, many referrals involve women with only 2 losses, and we do not withhold evaluation or treatment until they have had a further loss. As noted recently by Petrozza, *et al*[28], controversy exists regarding how many pregnancy losses should occur before a diagnostic evaluation is considered. In addition, if we are to assume that it is the presence of aPL that influences the development of pregnancy-associated TE in women with RPL, rather than their history of pregnancy loss, then the number of prior losses, whether 2 or 3, is immaterial. Indeed, the only patient with a postpartum event in our prospective, observational study had a history of only 2 losses and did not satisfy classification criteria for APS.

We confirmed an association between aPL positivity and prematurity, and observed a very low rate of postpartum thrombosis in patients with RPL, aPL, and no history of TE. Only one (1.2%) of our aPL-positive patients had a TE during

or after pregnancy, and this occurred despite concurrent prophylactic anticoagulant therapy. We agree with Gates, *et al*[29], who stated that there is still insufficient evidence on which to base recommendations for thromboprophylaxis during pregnancy and the early postnatal period for women with aPL and no history of non-pregnancy-related TE. Our findings suggest that randomized clinical trials of anticoagulant therapy are urgently needed to establish evidence-based standard of care for patients with RPL, and in particular, for patients with RPL and aPL.

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REFERENCES

1. Love PE, Santoro SA. Antiphospholipid antibodies: anticardiolipin and the lupus anticoagulant in systemic lupus erythematosus (SLE) and in non-SLE disorders. Prevalence and clinical significance. Ann Intern Med 1990;112:682-98.
2. Kutteh WH. Antiphospholipid antibody-associated recurrent pregnancy loss: treatment with heparin and low-dose aspirin is superior to low-dose aspirin alone. Am J Obstet Gynecol 1996;174:1584-9.
3. Rai R, Cohen H, Dave M, Regan L. Randomized controlled trial of aspirin and aspirin plus heparin in pregnant women with recurrent miscarriage associated with phospholipid antibodies (or antiphospholipid antibodies). BMJ 1997;314:253-7.
4. Empson M, Lassere M, Craig JC, Scott JR. Recurrent pregnancy loss with antiphospholipid antibody: a systematic review of therapeutic trials. Obstet Gynecol 2002;99:135-44.
5. Derksen RHWM, Khamashta MA, Branch DW. Management of the obstetric antiphospholipid syndrome. Arthritis Rheum 2004;50:1028-39.
6. Triolo G, Ferrante A, Ciccia F, et al. Randomized study of subcutaneous low molecular weight heparin plus aspirin versus intravenous immunoglobulin in the treatment of recurrent fetal loss associated with antiphospholipid antibodies. Arthritis Rheum 2003;48:728-31.
7. Derksen RHWM, de Groot PG, Nieuwenhuis HK, Christiaens GCML. How to treat women with antiphospholipid antibodies in pregnancy? Ann Rheum Dis 2001;60:1-3.
8. Geis W, Branch DW. Obstetric implications of antiphospholipid antibodies; pregnancy loss and other complications. Clin Obstet Gynecol 2001;44:2-10.
9. Lima F, Khamashta MS, Buchanan NM, et al. A study of sixty pregnancies in patients with the antiphospholipid syndrome. Clin Exp Rheumatol 1996;14:31-6.
10. Branch DW, Silver RM, Blackwell JL, et al. Outcome of treated pregnancies in women with antiphospholipid syndrome: An update of the Utah experience. Obstet Gynecol 1992;80:614-20.
11. Salonen Ros H, Lichtenstein P, Bellocco R, Petersson G, Cnattinguius S. Increased risks of circulatory diseases in late pregnancy and puerperium. Epidemiology 2001;12:456-60.
12. Clark CA, Spitzer KA, Nadler JN, Laskin CA. Preterm deliveries in women with systemic lupus erythematosus. J Rheumatol 2003;30:2127-32.
13. Khamashta MA, Cuadrado MJ, Mujic F, Taub NA, Hunt BJ, Hughes GRV. The management of thrombosis in the antiphospholipid-antibody syndrome. N Engl J Med 1995;332:993-7.
14. Kearon C, Ginsberg JS, Gent M. Anticoagulation for venous thromboembolism. N Engl J Med 1999;341:539-40.
15. Erkan D, Merrill JT, Yazici Y, Sammaritano L, Buyon JP, Lockshin MD. High thrombosis rate after fetal loss in antiphospholipid syndrome: effective prophylaxis with aspirin. Arthritis Rheum 2001;44:1466-9.
16. Pettila V, Kaaja R, Leinonen P, Ekblad U, Kataja M, Iddia E. Thromboprophylaxis with low molecular weight heparin (daleparin) in pregnancy. Thromb Res 1999;96:275-82.
17. Carson SA, Branch DW. Management of recurrent early pregnancy loss. ACOG Practice Bulletin 2001;24:372-83.
18. Backos M, Rai R, Baxter N, Chilcott IT, Cohen H, Regan L. Pregnancy complications in women with recurrent miscarriage associated with antiphospholipid antibodies treated with low dose aspirin and heparin. Br J Obstet Gynecol 1999;106:102-7.
19. Esplin MS. Management of antiphospholipid syndrome during pregnancy. Clin Obstet Gynecol 2001;44:20-8.
20. Rai R. Obstetric management of antiphospholipid syndrome. J Autoimmun 2000;15:203-7.
21. Toglia MR, Weg JG. Venous thromboembolism during pregnancy. N Engl J Med 1999;335:108-14.
22. Spitzer KA, Murphy K, Crowther M, Clark CA, Laskin CA. Post-partum management of women at increased risk of thrombosis — results of a Canadian pilot survey. J Rheumatol 2006;33:2222-6.
23. Hunt BJ, Gattens M, Khamashta M, Nelson-Piercy C, Almeida A. Thromboprophylaxis with unmonitored intermediate-dose low molecular weight heparin in pregnancies with a previous arterial or venous thrombotic event. Blood Coagul Fibrinolysis 2003;14:735-9.
24. Witlin AG, Mattar FM, Saade GR, Van Hook JW, Sibai BM. Presentation of venous thromboembolism during pregnancy. Am J Obstet Gynecol 1999;181:1118-21.
25. Brill-Edwards P, Ginberg JS, Gent M, et al. Safety of withholding heparin in pregnant women with a history of venous thromboembolism. N Engl J Med 2000;16:1439-44.
26. Clark CA, Spitzer KA, Nadler JN, Laskin CA. Preterm deliveries in women with systemic lupus erythematosus. J Rheumatol 2003;30:2127-32.
27. Kozer E, Costei AM, Boskovic R, Nulman I, Nikfar S, Koren G. Effects of aspirin consumption during pregnancy on pregnancy outcomes: meta analysis. Birth Defects Res B Dev Reprod Toxicol 2003;68:70-84.
28. Petrozza JC, Robertson AD, O'Brien B. Early pregnancy loss [monograph on the Internet]. eMedicine [updated 29 August 2006]. Available from: http://www.emedicine.com/med/topic3241.htm [accessed February 8, 2007].
29. Gates S, Brockelhurst P, Davis LJ. Prophylaxis for venous thromboembolic disease in pregnancy and the early post-natal period. Cochrane Database Syst Rev 2002;2:CD001689.

Low Molecular Weight Heparin and Aspirin for Recurrent Pregnancy Loss: Results from the Randomized, Controlled HepASA Trial

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ABSTRACT. ***Objective.*** To compare live birth rates in women with recurrent pregnancy loss (RPL) and either autoantibodies or a coagulation abnormality, treated with low molecular weight heparin plus aspirin (LMWH/ASA) or ASA alone, and to place our results in context with other randomized clinical trials (RCT) with similar cohorts.

Methods. The HepASA Trial was an RCT including patients with a history of RPL and at least 1 of the following: antiphospholipid antibody (aPL), an inherited thrombophilia, or antinuclear antibody. Treatment groups were stratified by aPL status and history of early versus late pregnancy losses. Patients received either LMWH/ASA or ASA alone. The primary outcome was live birth; secondary outcomes included adverse events and bone loss at the spine and femoral neck. Literature over the past 20 years was reviewed to identify comparable RCT.

Results. Over 4 years, 859 women with RPL were screened: 88 (10.2%) fulfilled inclusion criteria, became pregnant and were randomized to receive either LMWH/ASA or ASA alone. aPL were present in 42 (47.7%) patients in each group. The trial was stopped after 4 years when an interim analysis showed no difference in live birth rates in the 2 groups, and a lower rate of pregnancy loss in the ASA only group than expected. In the LMWH/ASA group, 35/45 (77.8%) had a live birth versus 34/43 (79.1%) in the ASA only group ($p = 0.71$). Neither number of prior losses nor aPL status was correlated with pregnancy outcome. There were no cases of pregnancy related thrombosis in either group. Mean change in BMD did not differ by treatment group at either the lumbar spine ($p = 0.57$) or femoral neck ($p = 0.15$). RCT since 2000 for aPL positive women with RPL and similar inclusion criteria report a mean live birth rate of 75% with either LMWH or ASA.

Conclusion. LMWH/ASA did not confer incremental benefit compared to ASA alone for this population. Regardless of treatment regimen, number of prior losses, or aPL positivity, almost 80% of women in our RPL cohort had a successful pregnancy outcome. These findings contribute to a growing body of literature that contests the emerging standard of care comprising LMWH/ASA for this population. (First Release Feb 1 2009; J Rheumatol 2009;36:279–87; doi:10.3899/jrheum.080763)

Key Indexing Terms:
RECURRENT PREGNANCY LOSS
RANDOMIZED CLINICAL TRIAL
LOW MOLECULAR WEIGHT HEPARIN
ANTIPHOSPHOLIPID ANTIBODIES

Approximately 15% of all clinically recognizable pregnancies end in pregnancy loss[1,2], and it has been estimated that recurrent pregnancy loss (RPL) affects up to 5% of couples trying to conceive[3]. A patient who has had 2 or more consecutive losses can be classified with RPL[4]. Possible causes to consider in evaluating RPL include anatomical, genetic, and hormonal factors, but in over 50% of cases no etiology is found[5]. Pregnancy loss in patients with antiphospholipid antibodies (aPL) including anticardiolipin (aCL) and the lupus anticoagulant (LAC) has been attributed to placental infarcts and vascular thrombosis leading to placental insufficiency[6,7]. Others have observed an inhibition of trophoblast invasion and differentiation as a possible mechanism for early pregnancy loss[8,9]. The role of an immune-mediated mechanism to explain RPL has been examined based on observations initially made in women with systemic lupus erythematosus (SLE), an autoimmune disease that can be accompanied by higher than normal rates of fetal loss[10]. Certainly, the deposition of immunoglobulins, com-

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plement, anti-DNA antibodies, and antinuclear antibodies (ANA) in the lupus placenta suggest that coagulopathy is not the only mechanism of placental (and therefore fetal) ischemia, and that immune-mediated inflammatory mechanisms may also contribute to intrauterine growth restriction (IUGR) and fetal loss[11].

The present standard of care for women with aPL and RPL is treatment with heparin and aspirin (ASA)[12]. Early data supporting the use of ASA and unfractionated heparin to improve live birth rate in women with RPL and aPL appeared in 2 small studies in the 1990s[13,14]. Rai and colleagues[14], in determining the required sample size for their randomized trial, predicted rates of loss with ASA alone that were significantly higher than observed by others both before and since[15-17]. They included women with very low levels of aPL, and found a decrease in early fetal loss with the use of unfractionated heparin and ASA compared to ASA only. They also reported no treatment benefit for pregnancies that survived beyond 13 weeks' gestation, a finding not supported by others[18]. In a more recent randomized clinical trial (RCT), Farquharson, *et al* found no improvement in pregnancy outcomes of aPL positive women with a history of RPL when comparing treatment with low molecular weight heparin (LMWH)/ASA vs ASA alone[17] and Carmona, *et al* found that preconceptual ASA treatment was an independent predictor of live birth[19]. Subsequent to the design and initiation of our HepASA trial, Empson, *et al*[20], in a Cochrane database review, concluded that treatment with unfractionated heparin and ASA may reduce pregnancy loss by 54% but that large randomized controlled trials are still needed to explore potential differences between unfractionated heparin and LMWH.

A relationship between inherited and acquired thrombophilias and RPL has been examined, with small uncontrolled studies favoring treatment with LMWH to improve pregnancy outcome[21-23], but evidence supporting this treatment remains inadequate[24]. A recent systematic review concluded that owing to limited evidence of efficacy, treatment with LMWH/ASA remains empiric in women with inherited thrombophilias[25].

As previous studies of these therapeutic regimens have not proven conclusive due to small sample sizes and/or weak study design, we undertook a randomized controlled trial of LMWH and ASA versus ASA alone for women with prior adverse obstetric outcomes, autoantibodies including aPL, and inherited thrombophilias, commencing randomization early in the first trimester. The primary objective of the HepASA Trial was to investigate whether treatment with LMWH plus ASA results in an increased rate of live births compared to treatment with ASA alone. The secondary objective was to compare adverse events and the incidence of bone loss in the 2 groups. In addition, we wanted to place our findings in the context of other comparable trials and determine why, in the apparent absence of consistent findings, a standard of care has emerged for this population.

MATERIALS AND METHODS

Objectives. In this open label RCT, we investigated whether treatment with LMWH/ASA results in increased live births in women with a history of consecutive RPL and autoantibodies compared to treatment with ASA alone.

Participants. Inclusion criteria: Women considered for participation in this trial fulfilled the following criteria: aged 18–44 years at the time of randomization; a history of ≥ 2 unexplained consecutive pregnancy losses prior to 32 weeks gestation; presence of at least 1 of the following: ANA, aPL, or an inherited thrombophilia; and confirmed pregnancy by either 2 appropriately rising quantitative beta human chorionic gonadotropin (βhCG) tests performed 48 h apart or by ultrasound confirming fetal heart activity.

Exclusion criteria: Exclusion criteria included SLE (fulfilling American College of Rheumatology classification criteria[26]), known peptic ulcer disease (within the last 5 yrs), sensitivity to ASA or heparin obtained by self report, bone mineral density z score < –2.5, known platelet function abnormality, or a previous thromboembolic event (TE) requiring ongoing anticoagulant therapy including heparin, ASA, or warfarin, verified in medical records. Patients were also excluded if any genetic, anatomic, or hormonal etiology for pregnancy loss was identified by (respectively) karyotype analysis of both partners, hysterosalpingogram/sonohystogram, and a hormonal evaluation (which included either an endometrial biopsy or loss while taking progesterone or clomiphene therapy or mid luteal phase serum progesterone levels timed appropriately). Any such patient was referred for appropriate counseling or treatment. Geographic distance from the clinic and hospitals in Toronto/Hamilton, Ontario, as well as failure to consent to participate were additional exclusion criteria.

Laboratory evaluation: Positive serology was defined as at least one of the following tests found to be positive on 2 occasions at least 8 weeks apart: anticardiolipin IgG [> 15 IgG phospholipid units (GPL)] or IgM [> 25 IgM phospholipid units (MPL)]; LAC measured by Russell's viper venom time (RVVT), kaolin-cephalin clotting time (KCCT), dilute prothrombin time (DilPT), or lupus sensitive activated partial thromboplastin time (aPTT-LA)[27]; or the presence of 1 positive test on a thrombophilia screen [which included protein C (functional), protein S (free) antigen functional, activated protein C resistance (APCR) assay, factor V Leiden, prothrombin 20210 AG, and methylenetetrahydrofolate reductase (MTHFR)]; or ANA ≥ 1/80. All eligible patients signed consent to participate at the pretrial visit and again at randomization once confirmed pregnant.

Clinical assessments: A study physician evaluated all randomized women every 6 weeks during their pregnancy. The assessment included a general examination preceded by a detailed history including a functional inquiry assessing for the presence of a systemic autoimmune disorder. Platelets were measured 6 to 12 days after beginning LMWH and every 6 weeks thereafter. Partial thromboplastin time (PTT) was measured at each visit. Obstetrical evaluations were performed according to accepted standard of practice. Data were collected at the obstetrical visits to document fetal development and well-being as well as maternal complications. Delivery information was obtained from the obstetrician or from medical records. Patients were seen 6 weeks post partum and had a bone mineral density (BMD) performed 6-12 weeks' post partum. Spine (L2–L4) and hip (femoral neck) BMD were measured in g/cm^2 by dual energy x-ray absorptiometry using a Lunar DPX-L bone densitometer (Lunar Corporation, Madison, WI, USA). Measurements throughout the trial were performed by a single, certified densitometry technician. The coefficient of variation determined by test-retest with repositioning was 1.18% at the spine and 1.56% at the femoral neck.

Adverse events: Side effects were recorded at each visit by the study physician using a standard questionnaire. BMD was assessed prepregnancy

and 6–12 weeks at a single laboratory by a single technician to allow quantification of the effect of LMWH on BMD as well as to determine the effect of pregnancy on BMD in the ASA only group.

Interventions/trial products. The trial drug, LMWH (Fragmin, dalteparin sodium), was provided by Pfizer (formerly Pharmacia and Upjohn) as a solution for injection with potency described in international anti-Xa units (IU) and supplied in multidose vials (25,000 anti-Xa IU/ml). All patients randomized to receive LMWH were taught to self-inject the medication subcutaneously once daily until 35 weeks' gestation or delivery, at a dose of 5000 IU/day. ASA (81 mg enteric coated) was used in combination with LMWH in the investigational group, and ASA alone was administered in the reference group. Medication compliance was measured at each clinical visit by patient self-report and return of used LMWH vials.

Sample size. The sample size estimate was based upon an expected live birth rate of 50% in the ASA-treated patients derived from the live birth rate in the placebo arm of the ASA/P study[15]. A 25% absolute improvement in live birth rate with LMWH/ASA treatment was hypothesized[14]. The sample size was based on the number needed to show a significant difference in proportions, with a 2-sided alpha of 0.05 and a beta of 0.10, and was calculated to be 90 patients per group (180 patients in total). We assumed a 10% dropout rate, resulting in a planned sample size of 200 with an accrual rate of 50 per year for the 4-year funding period. An interim analysis was built into the study design to occur once 90 patients were randomized.

Randomization. The randomization schedule was created by the Coordinating and Methods Centre (CMC) of McMaster University in Hamilton. Randomization was performed at the CMC, with communication by telephone, when an eligible patient was confirmed pregnant. Patients were randomized to receive prophylactic doses of LMWH and ASA or ASA only. The CMC assigned a study number to the patient and provided treatment allocation data to the study coordinator. Patients were stratified by presence or absence of aPL, and early (≤ 14 wks) versus late (15–32 wks) losses. The rationale for stratification was to enable subgroup analysis of aPL positive women, who may have a different risk or poorer outcomes than the other women included in this study. Women with a history of both early and late losses were assigned to the late stratum.

Blinding. The trial was an open label design, as the investigators did not consider daily subcutaneous placebo injections to pregnant women ethical; further, ascertainment of the primary endpoint (live birth) should not be subject to bias as a result of knowledge of treatment strategies. We also examined safety variables including adverse events and changes in BMD.

Statistical analysis. The primary analysis was "intent to treat" based on stratified logistic regression with an adjustment for age and timing of losses as well as the treatment variable. The trial design planned for a multivariate analysis, permitting subgroup analysis paying attention to antibody profiles, early and late losses, maternal age, and side effect profiles. Mean change in BMD from baseline to followup was compared by treatment group using a Student's t test. Participants were classified as having experienced bone loss at the spine and femoral neck separately based on the estimated least significant change. Logistic regression was used to examine the effect of treatment group on BMD loss for the spine and femoral neck separately. Statistical analysis was performed using SAS (Version 9.1).

Ethics approval. Our study was approved by the University of Toronto Ethics Review Board, Toronto, and by the Ethics Review Board at McMaster University (ClinicalTrials.gov registration number NCT00564174).

RESULTS

A total of 859 women were screened for inclusion in the HepASA trial between 2000 and 2004 (Figure 1). From the panel of laboratory markers tested, 266 women (31%) had at least 1 positive result. One hundred eighty-three of the positive patients completed the required gynecologic tests, and 112 of these patients consented to participate once they became pregnant. Twenty-four failed to conceive during the study period and 88 patients were randomized (10.2% of the total screened): 43 patients to receive ASA only and 45 to receive LMWH/ASA.

Figure 1 summarizes study recruitment. Baseline patient characteristics are summarized in Table 1. There were no significant differences between the 2 groups in demographic characteristics, obstetric history, mean age at randomization (33.8 vs 34.6 yrs), or laboratory profile. Forty-two patients (47.7%) of those randomized were aPL positive (25 for IgG; 6 for IgM; 15 for LAC; and some patients for more than 1 aPL) and 22% had one of the panel of thrombophilia markers tested, all heterozygous.

Our study was designed to have a sample size of 200 patients and we had anticipated completion within the 4-year funding period. However, after 4 years of recruitment and screening 859 women, only 88 patients had been randomized (Figure 1). The steering committee decided to end the trial upon completion of the 4-year funding period when it was revealed that an interim analysis showed no significant difference in pregnancy outcome between the groups. In addition, the pregnancy loss event rate in the ASA only group was much lower than originally hypothesized.

There were 35 (77.8%) live births in the LMWH/ASA group and 34 (79.1%) live births in the ASA only group ($p = 0.75$ by Fisher's exact test, Table 2). There was no association between number of previous pregnancy losses (2 vs ≥ 3) and pregnancy outcome: relative risk (RR) 0.70; 95% confidence interval (CI) of RR: 0.40–1.22 ($p = 0.20$). Mode of delivery was similar in the 2 groups: RR 1.04; 95% CI 0.77–1.41 ($p = 0.80$). One patient in the ASA only group had a twin pregnancy that resulted in 1 live birth and 1 stillbirth. Thus the total number of outcomes in the ASA only group was 44. A neonatal death occurred in the ASA group, attributed to cervical incompetence, preterm premature rupture of membranes, and delivery at 22 weeks' gestation (with the neonate living for less than 1 h). Spontaneous abortions ≤ 14 weeks' gestation occurred in 7 (15.5%) of the LMWH/ASA pregnancies and in 8 (18.6%) of the ASA only pregnancies with the mean (range) gestational age at the time of loss being 9.8 (7.7–10.7) weeks versus 8.9 (5.9–13.0) weeks, respectively. There were no pregnancy losses between 15 and 20 weeks' gestation.

The median birth weight in the LMWH/ASA group was 3404.5 grams (g) and 3250 g in the ASA only group ($p = 0.627$). Birth weights were below the 10th percentile based on Canadian census data[28] in 9 patients (including 1 twin stillbirth), with 3 in LMWH/ASA group and 6 in the ASA only group. Placental maturation was noted in one of these patients and increasing blood pressure in another, both resulting in the decision to induce labor. Obstetric histories of early versus late loss were not associated with any difference in the live birth rate in the 2 groups (Figure 2). Of the 11 patients with a history of stillbirth, none had a late preg-

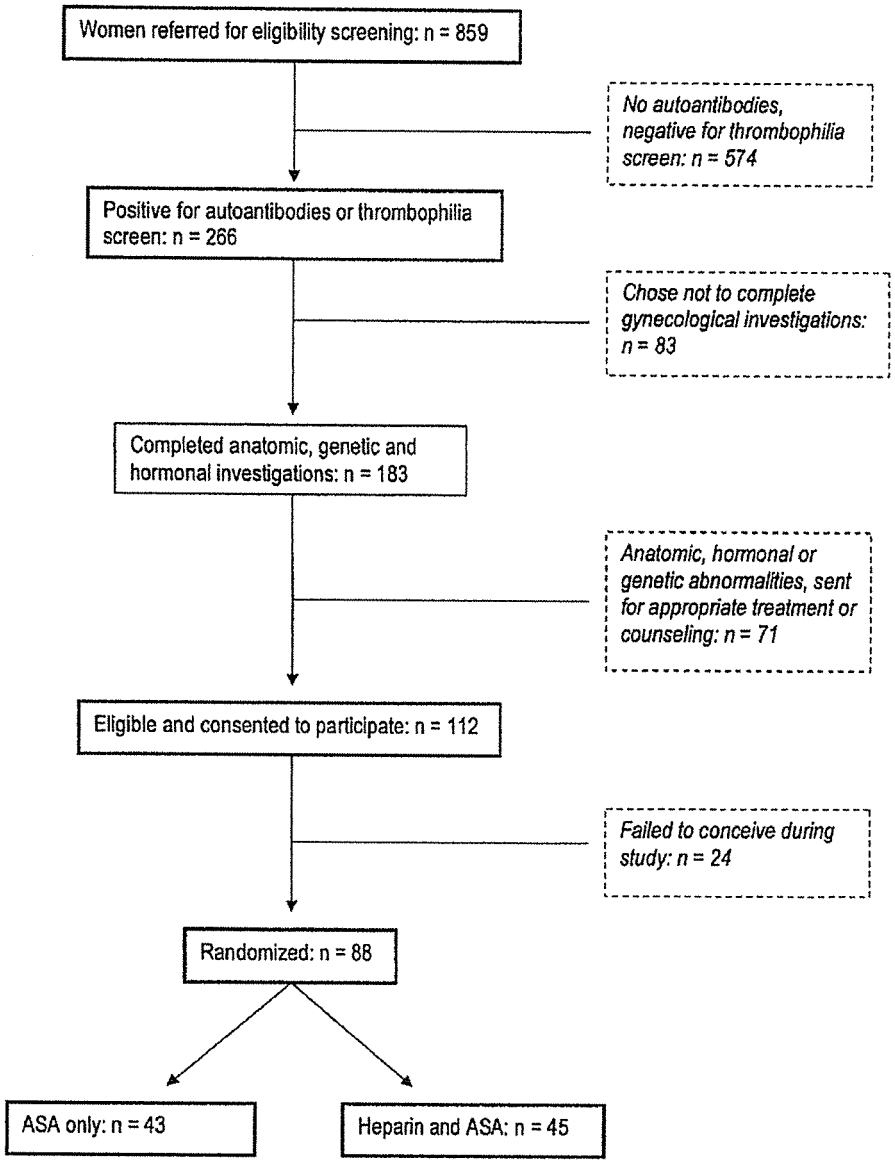

Figure 1. CONSORT flow chart for recruitment to the HepASA trial.

nancy loss or stillbirth in the study pregnancy: 7 had a live birth; 4 had early pregnancy losses. Three of the 7 live births occurred among the 9 patients with a birthweight less than the 10th percentile.

Stratification by presence or absence of aPL at randomization resulted in 22 aPL positive patients randomized to LMWH/ASA (48.9%) and 20 randomized to ASA (46.5%) (Table 1). Table 3 summarizes pregnancy outcome based on aPL presence and treatment group. No significant difference was detected with respect to pregnancy outcome. Live births were evenly distributed across GPL and MPL titers in both treatment groups, but the sample sizes were too small to enable any conclusions regarding the significance of that observation. There were no cases of a maternal thromboembolic event either during pregnancy or in the postpartum period in either group.

Of the 88 trial participants, 23/45 randomized to LMWH/ASA and 15/43 randomized to ASA alone completed both baseline and followup BMD assessments. For these 38 women, the mean duration of time between assessments

Table 1. Baseline patient characteristics of the 2 treatment groups.

Variable, n	ASA Only (n = 43)	LMWH/ASA (n = 45)
History of early losses (≤ 14 wks)	34	32
History of late losses (> 14 wks)	9	13
Still birth ever (20–32 wks)	7	4
Live birth ever	15	15
Maternal age at randomization, yrs, mean (SD)	33.8 (4.1)	34.6 (3.9)
Gestational age at randomization, wks, mean (SD)	5.6 (1.5)	5.8 (1.5)
Current smoker	3	5
aPL (aCL and/or LAC)	20	22
Inherited thrombophilia	10*	9**
ANA	15	15
≥ 2 Laboratory abnormalities	4	7

ASA: aspirin; LMWH: low molecular weight heparin; aPL: antiphospholipid antibody; aCL: anticardiolipin antibody; LAC: lupus anticoagulant; ANA: antinuclear antibody; SD: standard deviation. * 7 heterozygous for Factor V Leiden, 3 heterozygous for prothrombin 20210 AG. ** 7 heterozygous for Factor V Leiden, 1 heterozygous for prothrombin 20210 AG, and 1 heterozygous for both prothrombin 20210 AG and methylenetetrahydrofolate reductase (MTHFR).

Table 2. Pregnancy outcome stratified by treatment group. Data are presented as n (%). There were no statistically significant differences between the 2 treatment groups with regard to obstetrical outcomes. There was 1 twin pregnancy in the ASA only group, resulting in 44 pregnancy outcomes from 43 pregnancies.

Pregnancy Outcome, n	LMWH/ASA, n = 45	ASA Only, n = 43
Loss, ≤ 14 wks gestation	7 (15.6)	8 (18.6)
Loss, 14 to 20 wks gestation	0 (0)	0 (0)
Ectopic pregnancy	2 (4.4)	0 (0)
Still birth (20–32 wks)	1 (2.2)	1* (2.3)
Live birth	35 (77.8)	34* (79.1)
Induced delivery	9 (20.0)	8 (18.2)
Caesarian section	11 (24.4)	14 (31.8)
Neonatal death	0 (0)	1
Total	45	44*

* One patient had a twin birth with 1 still birth and 1 live birth. LMWH: low molecular weight heparin; ASA: aspirin.

was 1.35 years (0.84–2.82 yrs) and was similar for the 2 groups (p = 0.18). Mean change in BMD for the 38 women was –0.05 g/cm^2 at the spine (range –0.30 to 0.07) and –0.01 g/cm^2 at the femoral neck (range –0.16 to 0.115). Mean change in BMD did not differ by treatment group at either the lumbar spine (p = 0.57) or femoral neck (p = 0.15). Twenty-eight of the 38 women with complete BMD data experienced significant loss of bone mass over the intervening period at 1 or both of the lumbar spine or femoral neck; 27 of the 28 experienced loss at the spine, and 13 of the 28 experienced loss of bone mass at the femoral neck. The odds of loss of bone mass were not significantly different for those in the LMWH/ASA group compared with those in the ASA alone group [odds ratio, OR (any loss) = 0.97, 95% CI 0.22 – 4.24, p = 0.97; OR (spine loss) = 0.71, 95% CI 0.17–2.92, p = 0.63; and OR (hip loss) = 2.33, 95% CI 0.57–9.29].

DISCUSSION

The HepASA trial, comparing LMWH/ASA and ASA alone in women with RPL, did not show any demonstrable difference in the rate of live births in the 2 groups. Our results add to the body of literature[16,17] suggesting that use of heparin plus ASA in women with RPL may not be as beneficial as originally suggested[13,14]. There were no major adverse events associated with either treatment. One stillbirth did occur in each treatment arm and the only neonatal death occurred in the ASA only group, associated with cervical incompetence and preterm premature rupture of membranes at 22 weeks' gestation.

Two subgroups included in HepASA have been the subject of much discussion. The study was stratified by presence of aPL, as this population has been included in many previous studies. Although our final sample size resulted in lower than the desired statistical power, the subgroup of women with aPL did not appear to benefit from the use of LMWH plus ASA (Table 3). Unfortunately, although aCL titers and live births in our aPL positive women were evenly distributed between the 2 treatment groups, the sample size was not sufficient to enable any conclusions to be drawn with regard to titer-based treatment efficacy. Of patients screened for this trial, the number positive for aPL was 31% (256/859), which was lower than our previous experience[15], and as not all of these women satisfied our inclusion/exclusion criteria, we concur with the findings that approximately 20% of women with RPL have aPL[29].

The data collected in this study did not show evidence that LMWH use was associated with increased bone loss versus the ASA group. The safety of LMWH in pregnancy has been described by others[30,31] and bone loss appears to be similar for women on LMWH or on unfractionated heparin[32]. Both pre- and post-BMD measurements were obtained in 54% (38/70) of the patients with live births. We surmise that as our patients come from a wide radius around Toronto, the followup BMD appointment at the reference laboratory, located downtown, may have been difficult to attend despite our efforts to accommodate schedules. Additionally, since a number of our patients became pregnant very quickly after their successful pregnancy in this trial, they may not have undergone postpartum BMD if they were already pregnant.

We included women with a history of 2 or more consecutive first trimester pregnancy losses, as we and others have noted that the causes of RPL are similar in women with 2 or 3 losses[33]. We support investigation and, if appropriate, treatment of women with RPL after 2 consecutive losses rather than after a third loss[4]. Indeed, our analysis of women

ed to end the trial at the end of the 4-year funding period. The pregnancy loss event rate was much lower in the ASA only group than originally hypothesized and there was no difference in live birth rate in the 2 treatment groups. It was clear that a much expanded sample size would be necessary to detect a difference between the 2 arms and would require screening thousands more patients.

Our experience highlights the difficulty in recruiting RPL patients with autoantibodies and inherited thrombophilias into clinical trials and suggests that investigators studying this population need to carefully consider feasibility and multicenter collaboration. Despite the high referral rate to our center (> 200 patients/yr), it was not feasible to fulfill enrollment in an appropriately-powered decisive trial in 4 years. We recognized the need for collaborators early on but it proved difficult to find centers willing to participate. Some potential collaborators who treat women with RPL were uncomfortable with the trial inclusion of an ASA only treatment arm, as they felt it inappropriate to withhold heparin from aPL- or inherited thrombophilia-positive patients. Literature available at the time of trial design suggested a much higher incidence of inherited and acquired thrombophilias in women with RPL[19] than we have subsequently reported[36]. Although the association of thrombophilias with RPL has been confirmed in 2 metaanalyses[37,38] data supporting recommendations to treat with anticoagulants remain limited[24], and the small sample size of patients with thrombophilias in each of our study groups did not permit statistically valid comparison of treatment efficacy.

There have been a number of RCT for patients with RPL with aPL evaluating either unfractionated (UFH) or LMWH (Table 4) over the past 15 years. Each trial determined its own aPL inclusion criteria; for example, cutoffs for aCL IgG levels varied from a low of 5[14] to a high of 30[39] GPL, and each center measured LAC by different methodologies, and intuitively, these design inconsistencies might be expected to result in differential outcomes. However, regardless of differences in aPL status among the various trials, the live birth rates, whether in the UFH or LMWH treatment groups, are similar, ranging from 71.1% to 84%, with a weighted mean of 77.0% (standard deviation: 4.1). The only significant differences among trial outcomes are in the ASA-only treatment arms (Table 5): the live birth rates in those vary from a low of 42.2%[14] to a high of 80.0%[16]. Interestingly,

Table 4. Comparison of live birth rates for women with recurrent pregnancy loss and aPL. Results from 8 randomized clinical trials from 1996 to 2008 evaluating either unfractionated heparin or low molecular weight heparin in combination with aspirin.

Author, year	n	Determination of aPL Positivity	Intervention	Live Births (%)
Kutteh, 1996[13]	25	aCL IgG ≥ 27 or IgM ≥ 27 (Pos LAC excluded)	ASA 81 mg/day + sc UFH 5000 U bid	20/25 (80.0)
Rai, 1997[14]	45	aCL IgG > 5 or IgM > 3 or LAC (RVVT)	ASA 75 mg/day + sc UFH 5000 U bid	32/45 (71.1)
Cowchock, 1992[39]	26	aCL IgG > 30 or IgM > 11 or LAC (DRVVT or APPT)	ASA 80 mg/day + sc UFH 10,000 U bid	19/26 (73.1)
Noble, 2005[40]	25	aCL IgG > 20 or IgM > 20 or aPS > 3 MOM or LAC (DRVVT)	ASA 80 mg/day + sc UFH 5000 U bid	20/25 (80.0)
Franklin, 2002[41]	25	aCL IgG > 20 or IgM > 20 and/or aPS > 3 MOM and/or LAC (DRVVT)	ASA 81 mg/day + sc LMWH 5000 IU bid	19/25 (76.0)
Farquarson, 2002[17]	51	aCL IgG > 9 and/or IgM > 5 or LAC (DRVVT)	ASA 75 mg/day + sc LMWH 5000 IU/day	40/51 (78.4)
Noble, 2005[40]	25	aCL IgG > 20 and/or IgM > 20 and/or aPS > 3 MOM and/or LAC (DRVVT)	ASA 81 mg/day + sc LMWH 40 mg/day	21/25 (84.0)
Laskin, 2008	22	aCL IgG > 15 and/or IgM > 25 and/or LAC (DRVVT, PTTLA, DilPT, KCT)	ASA 81 mg/day + sc LMWH 5000 IU/day	17/22 (77.3)

RPL: recurrent pregnancy loss; UFH: unfractionated heparin; LMWH: low molecular weight heparin; ASA: aspirin; aPL: antiphospholipid antibody; aCL: anticardiolipin; LAC: lupus anticoagulant; DRVVT: dilute Russell's viper venom time; MOM: multiples of the mean; PTTLA: lupus anticoagulant sensitive partial thromboplastin time; DilPT: dilute prothrombin time.

Table 5. Comparison of studies with ASA only treatment arms for aPL positive patients with recurrent pregnancy loss.

Study, Year	n	aPL Positivity	% Live Births
Cowchock, 1992[39]	19	aCL IgG > 30 or IgM > 11 and/or LAC (DRVVT or APPT)	68.4
Kutteh, 1996[13]	25	aCL IgG ≥ 27 or IgM ≥ 27 (Pos LAC excluded)	44.0
Rai, 1997[14]	45	aCL IgG > 5 or IgM > 3 and/or LAC (RVVT)	42.2
Pattison, 2000[16]	20	aCL IgG ≥ 5 or IgM ≥ 5 and/or LAC (aPTT, DRVVT, KCT)	80.0
Farquarson, 2002[17]	47	aCL IgG > 9 or IgM > 5 and/or LAC (DRVVT)	72.3
Laskin, 2008	21	aCL IgG > 15 or IgM > 25 and/or LAC (DRVVT, PTTLA, DilPT, KCT)	71.4

ASA: aspirin; aPL: antiphospholipid antibody; aCL: anticardiolipin; LAC: lupus anticoagulant; DRVVT: dilute Russell's viper venom time; PTTLA: lupus anticoagulant sensitive partial thromboplastin time; DilPT: dilute prothrombin time.

both the lowest and the highest birth rates with ASA only treatment occurred in the trials with the lowest cutoff for aCL positivity, and the second lowest birth rate occurred in a trial that excluded LAC positive patients altogether[13]. The 2 trials with the lowest birth rates with ASA only treatment[13,14] were among the earliest completed with this population, and were also among those few that have reported a significant benefit for heparin and ASA over ASA alone. Their findings helped to establish the current standard of care that continues to recommend the use of LMWH/ASA for women with RPL and aPL.

However, the results from our HepASA trial add to a growing body of evidence reported since 2000[16,17,40,41] that does not support the use of LMWH plus ASA over ASA alone in this population. We agree with Gates, *et al*[42], who concluded after a thorough literature review, that for women with aPL, RPL, and no prior history of thrombosis, there is insufficient evidence to base recommendations for thromboprophylaxis in pregnancy.

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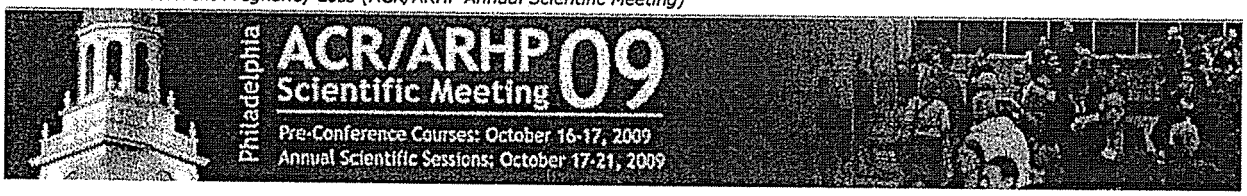
REFERENCES

1. Wilcox AJ, Weinberg CR, O'Connor JF, et al. Incidence of early loss of pregnancy. N Engl J Med 1988;319:189-94.
2. Orvieto R, Achiron A, Ben-Rafael Z, Achiron R. Intravenous immunoglobulin treatment for recurrent abortions caused by antiphospholipid antibodies. Fertil Steril 1991;56:1013-20.
3. Stephenson M, Kutteh W. Evaluation and management of recurrent early pregnancy loss. Clin Obstet Gynecol 2007:50:132-45.
4. Carson SA, Branch DW. ACOG Practice Bulletin 24. Management of recurrent early pregnancy loss. 2002 Compendium of Selected Publications. Washington, DC: American College of Obstetricians and Gynecologists; 2001:372-83.
5. Ramsay Goldman R. Pregnancy in systemic lupus erythematosus. Rheum Dis Clin North Am 1988;14:169-85.
6. Rand JH, Wu XX, Andree HAM, et al. Pregnancy loss in antiphospholipid antibody syndrome – a possible thrombogenic mechanism. N Engl J Med 1997;337:154-60.
7. De Wolf F, Carreras LO, Moerman P, et al. Decidual vasculopathy and extensive placental infarction in a patient with repeated thromoboembolic accidents, recurrent fetal loss and a lupus anticoagulant. Am J Obstet Gynecol 1982;142:829-34.
8. Sebire NJ, Fox H, Backos M, Rai R, Paterson C, Regan L. Defective endovascular trophoblast invasion in primary antiphospholipid antibody syndrome-associated early pregnancy failure. Hum Reprod 2002;17:1067-71.
9. Quenby S, Mountainfield S, Carthwright JE, et al. Antiphospholipid antibodies prevent extravillous trophoblast differentiation. Fertil Steril 2005;83:691-8.
10. Ramsay Goldman R. Pregnancy in systemic lupus erythematosus. Rheum Dis Clin North Am 1988;14:169-85.
11. Kitridou RC, Murphy Goodwin T. The fetus in systemic lupus erythematosus. In: Wallace DJ, Hahn B, Hahn BH, Dubois EL, eds. Systemic lupus erythematosus. Vol 7. Lippincott-Williams: 2006:1039-57.
12. Ruiz-Irastorza G, Khamashta MA, Hughes GR. Treatment of pregnancy loss in Hughes syndrome: a critical update. Autoimmun Rev 2002;1:298-304.
13. Kutteh WH. Antiphospholipid antibody-associated recurrent pregnancy loss: treatment with heparin and low dose aspirin is superior to low dose aspirin alone. Am J Obstet Gynecol 1996;174:1584-9.
14. Rai R, Cohen H, Dave M, Regan L. Randomized controlled trial of aspirin and aspirin plus heparin in pregnant women with recurrent miscarriage associated with phospholipids antibodies. BMJ 1997;314:253-7.
15. Laskin CA, Bombardier C, Hannah ME, et al. Prednisone and aspirin in women with autoantibodies and unexplained recurrent fetal loss. N Engl J Med 1997;337:148-53.
16. Pattison NS, Chamley LW, Birdsall M. Does aspirin have a role in improving pregnancy outcome for women with antiphospholipid antibody syndrome – A randomized clinical trial. Am J Obstet Gynecol 2000;183:1008-12.
17. Farquharson RC, Quenby S, Greaves M. Antiphospholipid syndrome in pregnancy: A randomized controlled trial of treatment. Obstet Gynecol 2002;100:408-13.
18. Jaffe R, Jauniaux E, Hustin J. Maternal circulation in the first trimester human placenta-myth or reality? Am J Obstet Gynecol 1997;176:695-705.
19. Carmona F, Font J, Azulay M, et al. Risk factors associated with fetal losses in treated antiphospholipid syndrome pregnancies: a multivariate analysis. Am J Reprod Immunol 2001;46:274-9.
20. Empson M, Lassere M, Craig J, Scott J. Prevention of recurrent miscarriage for women with antiphospholipid antibody or lupus anticoagulant. Cochrane Database Syst Rev 2005;CD002859.
21. Preston FE, Rosendaal FR, Walker ID, et al. Increased fetal loss in women with heritable thrombophilia. Lancet 1996;348:913-6.
22. Carp H, Dolitzky M. Inbal A. Thromboprophylaxis improves the live birth rate in women with consecutive miscarriages and hereditary thrombophilia. J Thromb Haemost 2003;1:433-8.
23. Brenner B, Bar J, Ellis M, et al. Effects of enoxaparin on late pregnancy complications and neonatal outcome in women with recurrent pregnancy loss and thrombophilia: results from the Live-Enox study. Fertil Steril 2005;84:770-3.
24. Middeldorp S. Thrombophilia and pregnancy complications: cause or association? J Thromb Haemost 2007;5 Suppl:276-82.
25. Di Nisio M, Peters L, Middeldorp S. Anticoagulants for the treatment of recurrent pregnancy loss in women without antiphospholipid syndrome. Cochrane Database Syst Rev 2005;18:2.
26. Tan EM, Cohen AS, Fries JF, et al. The 1982 revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 1982;25:1271-7.
27. Clark CA, Spitzer KA, Crowther MA, et al. Incidence of post partum thrombosis and preterm delivery in women with antiphospholipid antibodies and recurrent pregnancy loss. J Rheumatol 2007;34:992-6.
28. Kramer MS, Platt RW, Wen SW, et al. A new and improved population-based Canadian reference for birth weight for gestational age. Pediatrics 2001;108:E35.
29. Erkan D, Merrill JT, Yazici Y, et al. High thrombosis rate after fetal loss in antiphospholipid syndrome: effective prophylaxis with aspirin. Arthritis Rheum 2001;44:1466-7.
30. Carlin AJ, Farquharson RG, Quenby SM, Topping J, Fraser WD. Prospective observational study of bone mineral density during pregnancy: low molecular weight heparin versus control. Hum Reprod 2004;9:1211-4.
31. Roger MA, Kahn SR, Cranney A. Long-term dalteparin in pregnancy not associated with a decrease in bone mineral density: substudy of a randomized controlled trial. J Thromb Haemost 2007;5:1600-6.
32. Casele H, Haney EI, James A, Rosene-Montella K, Carson M. Bone density changes in women who receive thromboprophylaxis in

pregnancy. Am J Obstet Gynecol 2006;195:1109-13.
33. American College of Obstetricians and Gynecologists. Management of recurrent early pregnancy loss. ACOG Practice Bulletin No. 24. 2001;24:1-8
34. Petri M, Allbritton J. Fetal outcome of lupus pregnancy: a retrospective case-control study of the Hopkins Lupus Cohort. J Rheumatol 1993;20:650-6.
35. Hughes G. Hughes syndrome: the antiphospholipid syndrome – a clinical overview. Clin Rev Allergy Immunol 2007;32:3-12.
36. Spitzer, KA, Clark CA, Laskin AS, et al. Prevalence of inherited thrombophilias in women with unexplained recurrent pregnancy loss. Arthritis Rheum 2002;46 Suppl:S231.
37. Rey E, Kahn SR, David M, Shrier I. Thrombophilic disorders and fetal loss: a meta-analysis. Lancet 2003;361:901-8.
38. Kovalsky G, Garcia CR, Berlin JA, et al. Evaluation of the association between hereditary thrombophilias and recurrent pregnancy loss: a meta analysis. Arch Intern Med 2004;164:558-63.
39. Cowchock FS, Reece EA, Balaban D, Branch DW, Plouffe L. Repeated fetal losses associated with antiphospholipid antibodies: a collaborative randomized trial comparing prednisone with low-dose heparin treatment. Am J Obstet Gynecol 1992;166:1318-23.
40. Noble LS, Kutteh WH, Lashey N, Franklin RD, Herrada J. Antiphospholipid antibodies associated with recurrent pregnancy loss: prospective, multicenter, controlled pilot study comparing treatment with low-molecular-weight heparin versus unfractionated heparin. Fertil Steril 2005;83:684-90.
41. Franklin RD, Kutteh WH. Antiphospholipid antibodies (APA) and recurrent pregnancy loss: treating a unique APA positive population. Human Reproduction 2002;17:2981-5.
42. Gates S, Brockelhurst P, Davis LJ. Prophylaxis for venous thromboembolic disease in pregnancy and the early post natal period. Cochrane Database Syst Rev 2002;2:CD001689.

Presentation: Longterm Follow-up of ASA/P Trial Participants: No Evidence of Thrombotic Sequelae 20 Years After Anti-Phospholipid-Associated Recurrent Pregnancy Loss (ACR/ARHP Annual Scientific Meeting)



1283 - Longterm Follow-up of ASA/P Trial Participants: No Evidence of Thrombotic Sequelae 20 Years After Anti-Phospholipid-Associated Recurrent Pregnancy Loss

Tuesday, October 20, 2009: 9:00 AM - 11:00 AM
Hall D (Pennsylvania Convention Center)

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Presentation Number: 1283
Poster Board Number: 16

Purpose:

ASA/P (1988-94) was a double-blind, controlled, randomized therapeutic trial of 202 women with a history of recurrent pregnancy loss (RPL) with or without antiphospholipid antibodies (aPL). The study found no difference in live birth rates between treatment with either aspirin and prednisone (ASA/P) or placebo, between a history of early vs late losses, or between aPL positive or negative patients. Others have reported increased rates of thrombotic events (TE) and development of the antiphospholipid syndrome (APS) in this patient population in longterm followup studies 1-12 years after pregnancy loss accompanied by aPL. We wanted to determine the incidence of subsequent TE and APS in our longterm followup cohort.

Method: All participants in the ASA/P trial were sent questionnaires in 2008-09 that included a series of self-report yes/no questions based upon the clinical classification criteria for SLE/APS. We also requested information regarding subsequent pregnancies, medications currently being taken, and medical specialists currently being seen.

Results: Forty (19.8%) patients were considered lost to followup after their questionnaires were "returned-to-sender" and further attempts to update contact information failed. Fifty-five completed questionnaires were received (34.0% response rate). Median age of respondents: 51 (range 42-57); median years since trial participation: 17 (range 14-20). When comparing respondents to the original trial participants, 52% of respondents had received ASA/P, 67% had a live birth in the trial, and 51% were aPL-positive, not significantly different from the group as a whole. Twenty-three women had 34 subsequent pregnancies resulting in 22 live births, 10 early losses, 1 stillbirth, and 1 ectopic pregnancy (18/23 women had at least 1 live birth in the interval since trial participation). There have been no TE and no one has been diagnosed with APS.

Conclusion: The group of 55 women who responded to our followup questionnaire 14-20 years after participating in the ASA/P trial was representative of the original study cohort. In contrast to other longterm followups of patients with RPL and aPL, none of our respondents reported any TE and none has been diagnosed with APS. These results suggest that RPL, even in the context of aPL, is neither associated with nor predictive of the development of subsequent TE even as long as 20 years after initial presentation.

Keywords: pregnancy, randomized trials and thrombosis

Disclosure: C. A. Clark, None; **K. A. Spitzer**, None; **C. A. Laskin**, None.

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See more of: Antiphospholipid Syndrome: Pathogenesis and Clinical Aspects
See more of: ACR Poster Sessions

CHAPTER 53

c00053

Pregnancy and Autoimmune Rheumatic Disease

CARL A. LASKIN, CHRISTINE A. CLARK AND KAREN S. SPITZER

s0010

PREGNANCY AND THE RHEUMATIC DISEASES

p0030

Rheumatic diseases occur most often in women, ranging from an incidence of 9:1 (female:male) in systemic lupus erythematosus (SLE) to 3:1 in rheumatoid arthritis (RA). It follows logically that physiological states characterized by hyperestrogenicity would likely have an effect

p0040

on disease activity. Pregnancy may therefore exacerbate disease or be associated with disease amelioration, if not remission.[1] Medical issues associated with pregnancy may be rather complicated due to the multisystemic nature of rheumatic disorders,[2] presenting many challenges for the attending physicians, whether general internists, rheumatologists, or obstetricians. Of particular concern are the medications used to treat these patients. Although some medications may be completely safe in pregnancy, it may be necessary, depending on the drug, to discontinue certain agents from 3 weeks to 2 years prior to conception. Under ideal circumstances, any patient with an underlying medical problem should be assessed prior to a pregnancy in order that the pregnancy can be undertaken electively and as safely as possible. On too many occasions a patient may first present in a medical office pregnant either with active disease or on unsafe medications. Critical decisions must be made at these times that may be difficult emotionally and ethically.

p0020

Among the rheumatic diseases that could be addressed in this chapter, many are simply too uncommon to warrant detailed discussion. Therefore, those disorders that are more likely to be encountered by the clinician will be discussed.

IMMUNE FUNCTION IN THE CONNECTIVE TISSUE DISEASES AND PREGNANCY

Most rheumatic diseases are characterized by abnormalities in immunoregulation due to an imbalance in immune suppressor activity. Pregnancy and the accompanying hormonal changes may have a significant effect on disease manifestations, either amelioration or exacerbation.

Circulating autoantibodies are the primary marker of most connective tissue diseases (CTD), which may either be causative of or merely associated with tissue damage. These autoantibodies may bind with antigens on cell surfaces resulting in tissue destruction. As an alternative to this cytotoxic mechanism, the autoantibody may bind with antigen to form either a circulating or in situ immune complex or fix complement, thereby initiating an inflammatory response when fixed in tissues, which then results in damage (immune complex mechanism). The clinical manifestations vary according to the site of tissue injury, which, in turn, varies according to the disease entity. This may lead to inflammatory synovitis as in RA or a multisystem disorder as in SLE.[3]

p0050

During pregnancy a number of alterations in immune function occur affecting lymphocyte function, humoral immunity, and the inflammatory response. Awareness of these changes is of paramount importance in the assessment of a patient with a known or possible CTD. Human pregnancy is associated with an increase in immune suppressor activity leading to a decrease in humoral B cell function. In many autoimmune diseases there is dysregulation of the immune response leading to an impairment of suppressor activity resulting in polyclonal B cell activation. Therefore

the superimposition of pregnancy on the immunoregulatory abnormalities present in a woman with a CTD will alter the immune environment and may even correct the abnormalities existing in the CTD. Regardless, the interaction of these two altered immune states must be appreciated in order to account for changes in disease activity.

s0040

A BRIEF OUTLINE OF THE IMMUNOLOGY OF IMPLANTATION, PREGNANCY, AND LABOR AND DELIVERY

p0070

The fundamental theory of the establishment of pregnancy has been the Th1/Th2 paradigm.[1] Helper-T cell clones can be divided into two phenotypes that secrete a distinct cytokine pattern (Table 53.1).[4] During pregnancy, there is an overall suppression of Th1-mediated cellular immunity and an enhancement of Th2-mediated humoral immunity. The maintenance of pregnancy requires the downregulation of the pro-inflammatory Th1 cytokines (tissue necrosis factor-alpha [TNF-α], interferon gamma [IFN-γ], and interleukin-2 [IL-2]) and the upregulation of anti-inflammatory Th2 cytokines (IL-4, IL-6, and IL-10). During embryo implantation, uterine epithelial cells surrounding the blastocyst undergo apoptosis. These apoptotic cells are then taken up by macrophages found in excess at the implantation site. The uptake of the apoptotic cells into the macrophages promotes the secretion of Th2 cytokines and suppresses the release of Th1 cytokines including TNF-α from the macrophages. The Th2-rich anti-inflammatory environment surrounds and protects the developing embryo. Therefore pregnancy appears to be a Th2 phenomenon. Recent data, however, suggest that this paradigm may be overly simplistic. Although TNF-α and IFN-γ have been implicated in failed implantation or early pregnancy loss, it now appears that small quantities of these cytokines may be necessary for the successful implantation of the embryo. Moreover, it may be that Th1 activity both accompanies and predominates over Th2-mediated events during the early implantation period, and premature and term labor. Th1 activity plays an important role in the promotion of the Th2 response, regulation of the placentation process, defense against infections, and initiation of delivery. The new paradigm should more properly be thought of as "Th1–Th2 cooperation" rather than a Th2-dominant phenomenon.

t0010 TABLE 53.1 Distribution of cytokine production between helper cell clones, Th1 and Th2[4]

Th1	Th2
IFN-γ	IL-3
IL-2	IL-4
TNF-β	L-5
TNF-α	IL-6
GM-CSF	IL-10
IL-3	IL-13
IL-10	TNFα
	GM-CSF

IFN: interferon; IL: interleukin; TNF: tumor necrosis factor; GM-CSF:granulocyte macrophage colony-factor.

MANAGEMENT OF REPRODUCTIVE ISSUES IN THE RHEUMATIC DISEASES

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As with any woman with an underlying medical problem, pregnancy should ideally be elective. Planning minimizes the risks not only of disease complications to mother and fetus but also of birth defects due to medication. Prior to becoming pregnant, the patient should be assessed by an internist with specific notice taken of the manifestations of the underlying disease, the past history of exacerbations, and past and current medications. The attending physician will then be able to document (a) the clinical profile; (b) the laboratory profile; (c) the frequency and pattern of the most recent disease flare; and (d) the current medications.

p0060

- Clinical profile: This profile is constructed from the history and physical examination. In addition, the past history is utilized to record how the disease initially presented and the usual manifestations of a flare. u0010
- Laboratory profile: The laboratory tests that best reflect the patient's disease status are used as indicators of disease activity during a pregnancy. The profile is constructed from the current laboratory tests and any relevant serology, as well as noting which laboratory variables are associated with disease activity. u0020
- Most recent disease flare: It is important to determine and document the most recent exacerbation of the disease as well as the severity. The timing of a pregnancy will determine the safety of such an undertaking. Appropriate counseling of the patient can then be undertaken. This information should be incorporated into the patient's clinical profile. u0030
- Medications: A detailed history of current medications is essential to provide appropriate counseling to the patient regarding the timing of a pregnancy. Many antirheumatic drugs are unsafe in pregnancy, and because many are also long acting, they may have to be discontinued months prior to conception. u0040

SYSTEMIC LUPUS ERYTHEMATOSUS

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SLE is a multisystem autoimmune disorder characterized by diverse clinical and laboratory abnormalities, with a variable disease course. The disease predominantly affects young women in childbearing years but may occur in any

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s0070 p0170 age group. The prevalence has been reported from 15 to 50 per 100 000 population but varies considerably according to race and ethnic background. Clinical manifestations are the result of inflammation of multiple organ systems including skin, joints, kidneys, nervous system, and serosal membranes. Diagnosis and management of SLE is often difficult due to heterogeneity of the clinical and laboratory manifestations. Most clinicians rely upon the American College of Rheumatology (ACR) 1982 classification criteria to assist in the assessment of patients with an established or possible diagnosis of lupus.[5] Genetic factors have been documented with a strong association with MHC components HLA-DR2 and -DR3[6] p0180

Apart from the minor organ manifestations of arthritis and dermatitis, clinical evidence of renal involvement is found in 50–66% of patients with SLE. The World Health Organization has recognized six classes of lupus glomerulonephritis.[7] The prognosis varies according to the severity of involvement with mesangial and membranous glomerulonephritis having a better prognosis than proliferative disease. Central nervous system involvement may present with neurologic and/or psychiatric manifestations.

p0140 Laboratory findings in SLE include cytopenias, abnormal renal function tests, hypergammaglobulinemia, and a number of circulating autoantibodies. Among the most significant autoantibodies is anti-dsDNA antibody, which may be both pathogenic and a marker of disease activity. In addition, depressed complement levels correlate with disease activity.

s0060 Systemic Lupus and Fertility

p0150 Although studies indicate no difference in fertility rates in women with SLE compared to the general population, one must interpret these findings with caution.[8] Any active inflammatory disorder impacts the pituitary–ovarian axis leading to anovulation. In addition, the adverse effects of certain medications such as cyclophosphamide may severely compromise gonadal function. Therefore, under these circumstances, fertility rates will be affected.

p0160 There are women with well-controlled SLE who may require fertility treatment often in the form of ovulation induction therapy. This treatment requires the use of high doses of exogenous follicle stimulating hormone (FSH) leading to a hyperestrogenic state. The hyperestrogenicity may exacerbate lupus. There are several studies of ovulation induction therapy in women with SLE.[9–12] For the most part, the women have fared well although some complications associated with disease exacerbation have occurred, but since the studies are small, no conclusions can be drawn. Although such therapy is not contraindicated in women with SLE, it is advisable to initiate ovulation induction only in those individuals whose disease is under excellent control for an extended period such as 6 months. In addition, the patient must be assessed for disease activity and advisability of entering a pregnancy.

The Pre-Pregnancy Evaluation

Whenever possible, women with SLE contemplating pregnancy should be evaluated prior to conception for the status of their disease and its activity. The clinician should determine the patient's clinical and laboratory profile, date of most recent flare of the disease, and current medications. Using a methodical approach the physician may grasp the subtle nuances of the underlying disease permitting better distinction of disease activity and the physiological or pathophysiological changes associated with pregnancy.

Pregnancy in any woman with an underlying medical problem should be managed by both an internist/subspecialist and obstetrician/perinatologist. In the case of SLE, that internist should be a rheumatologist or at the very least an internist familiar with the management of lupus. The pre-pregnancy evaluation should be undertaken by an internist/rheumatologist and a recommendation made to the patient regarding the medical management plan. It is often necessary to delay a pregnancy until the disease comes under better control or while medications are changed to those more compatible with pregnancy. Appropriate counseling regarding the potential effect of the disease on the pregnancy and neonate, and similarly the effect of the pregnancy on the disease should be undertaken. p0130

The Effect of Pregnancy on SLE s0080

There is some consistency in the response of SLE to pregnancy but some patients do not "behave" as expected. As early as 1952, it was noted that some women with SLE flare during pregnancy.[13] Other studies have also noted an increase in flares during pregnancy.[14–17] The more recent literature, however, notes that the frequency of disease exacerbation during pregnancy and postpartum is less than that reported earlier.[18,19] In a case–control prospective study comparing pregnant and non-pregnant women with similar manifestations of SLE, Lockshin *et al.* found no increase in flares during pregnancy.[20,21] In contrast, Petri concluded that pregnancy was associated with an increased rate of disease flares in her population with a frequency of 1.63 per person years compared to 0.64–0.65 in a postpartum group or in non-pregnant controls.[22] Ruiz-Irastorza *et al.* observed findings similar to Petri with a 65% flare rate during pregnancy compared to 42% in the controls group.[23] Recent studies by Cortes-Hernandez *et al.* found that 33% of their lupus patients flared during pregnancy, with 26% in the second trimester and 51% post partum.[24] The major predictors of a flare were the discontinuation of antimalarial treatment, a history of more than three flares before the pregnancy, and a SLEDAI score (a standardized measure of lupus disease activity) ≥5 during these flares. In one study of 46 women with SLE who underwent 61 pregnancies, Urowitz *et al.* observed no increased frequency of lupus flares, using the SLEDAI, during pregnancy compared with controls.[25] p0190

Indeed there was a reduced chance of flare during a pregnancy if the patient had inactive disease for 6 months prior to conception. Clearly there is a lack of consensus among these studies. This may be due to dissimilar entry criteria, differing definitions of a flare, distinct patient populations, and differing control groups.[22,23]

s0100

The incidence of adverse pregnancy outcomes in women with SLE is increased. In a retrospective analysis, 555 women with SLE had an adverse outcome apart from manifestations of SLE compared to a group of 600000 controls.[26] These outcomes included hypertension, renal disease, preterm delivery, non-elective cesarean section, postpartum hemorrhage, and delivery-related deep venous thrombosis. Clark *et al.* noted 38.9% of women with SLE had a preterm delivery (before 37 weeks gestation) in a group of 72 pregnancies[27] The observation of preterm delivery was associated with disease activity and the presence of IgG anti-cardiolipin antibody.

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Renal disease may flare during a pregnancy, which may be related to insufficient steroid dosage in treatment.[23] Studies have been inconsistent in finding deterioration in renal function associated with pregnancy.[18,26,28–32] Tozman *et al.* noted no recurrence of renal disease in 11 of 18 patients with similar findings by Jungers *et al.* and Huong *et al.* All noted that the best prognosis was associated with remission of the disease at pregnancy onset. Furthermore, they all noted a higher risk of pre-eclampsia and premature birth than expected. In contrast, others noted an increase of renal flare in pregnancy.[30,31] These authors also conclude that the only predictor of a favorable maternal outcome in a pregnancy is quiescence of renal disease.

p0210

In contrast to renal disease, there is very little studied with respect to central nervous system (CNS) disease. Suffice it to say that moderate to severely active lupus is a high-risk situation for both mother and fetus.[31]

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Pre-eclampsia and Active SLE

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It is a major diagnostic challenge to distinguish pre-eclampsia from a lupus flare during pregnancy. Pre-eclampsia occurs in approximately 13% of lupus pregnancies and can be as high as 66% in those with renal disease.[21,33] Defining the clinical and laboratory profile of the patient before pregnancy will often assist in distinguishing these two conditions. If there are clinical features of active lupus and positive serology, this is likely a lupus flare. Depressed complement levels are characteristic of active lupus whereas elevated complement levels are seen in pregnancy and do not change in pre-eclampsia. Although proteinuria may be seen both in pre-eclampsia and lupus nephritis, the presence of an active sediment is a feature of active nephritis and not pre-eclampsia. Elevated liver function tests and uric acid with thrombocytopenia and decreased urinary excretion of calcium are characteristic of pre-eclampsia whereas thrombocytopenia alone or, if there is renal insufficiency, elevated serum urate can be seen in active SLE. Knowledge of your patient and how her disease has manifested itself in the past will assist immeasurably in correctly assessing this difficult diagnostic situation.

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Effect of SLE on Pregnancy

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Adverse pregnancy outcome is more common in SLE than in any other rheumatic disease. Appropriate pre-pregnancy evaluation and counseling maximizes the probability of a successful outcome for both the mother and the neonate. Maintaining the viability of the pregnancy requires close collaboration between the obstetrician/perinatologist and internist/rheumatologist.

Spontaneous Abortion, Prematurity, and Stillbirth

It has been reported in the past that the incidence of fetal wastage in SLE pregnancies approximated 50%, which included miscarriage, prematurity, and stillbirth.[14,17,22,23,25,34–36] However, a recent analysis of long-term data indicated that the spontaneous abortion rate in SLE has declined from 50% to less than 20% over the past 40 years (Figure 53.1).[37] Among the risk factors associated with adverse outcomes are antiphospholipid antibodies, hypocomplementemia, and hypertension during pregnancy.[24] Although some have noted that infants born to mothers with lupus are small for gestational age, others have not observed this even in cases where placental size is reduced.

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The increased frequency of fetal loss in SLE may be due to several factors: (i) active lupus resulting in decidual vasculitis which in turn compromises placental blood flow

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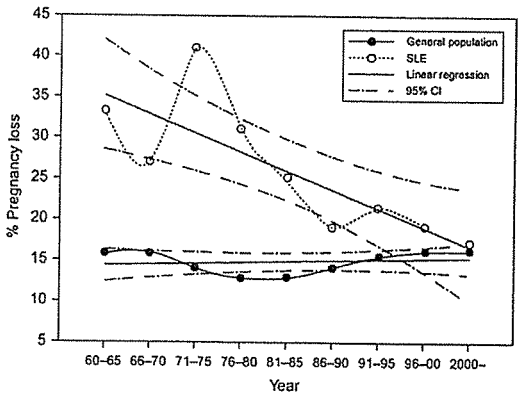

FIGURE 53.1 Change in rate of fetal loss in SLE pregnancies and in the US general population over the past 40 years.[37] Data were grouped into 5-year periods (except for the first period, 1963–1965, and the last period, 2000–2003).
Redrawn from Clark, Spitzer, and Laskin, 2005, Figure 1, p. 1710, by permission of the *Journal of Rheumatology*.

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depriving the fetus; (ii) trophoblast-reactive lymphocytotoxic antibodies; (iii) anti-Ro/SSA or anti-La/SSB antibodies with their associated compromise of the fetal cardiac conduction system; and (iv) antiphospholipid antibodies with resulting placental vascular thrombosis and insufficiency culminating in ischemic pregnancy loss.

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Antiphospholipid Antibodies and Pregnancy

p0300 Antiphospholipid antibodies (aPL) in low to moderate titer may be seen in 40–60% of lupus patients with active disease. The aPL family of antibodies includes IgG or IgM anti-cardiolipin antibodies (aCL) and a non-specific in vitro inhibitor of coagulation often referred to as the lupus anticoagulant (LAC). Although the mere presence of aPL in a woman with lupus may not be associated with any particular manifestation, there may be an increased risk of adverse pregnancy outcome in such individuals. A review of 10 studies comprising 554 women with SLE observed fetal loss more frequently in the presence of aPL (39–59%) compared to those without aPL (16–20%).[38] In addition, aPL have been associated with pre-eclampsia and placental abruption.[39–42] Women with SLE and aPL and the associated clinical manifestations including thromboembolism and pregnancy wastage, are classified with secondary antiphospholipid syndrome (APS). Those with only aPL and an associated clinical feature are classified as primary APS since they lack any other feature of SLE.

p0280 LAC and aCL have often been used interchangeably to indicate the presence of aPL. While there is certainly a correlation between these two antibodies, they are not identical. Lockshin *et al.* reported that while both the LAC and aCL were associated with fetal loss, higher levels of aCL appear to be more predictive of fetal distress or fetal death among pregnant women with SLE.[42] However, other recent observations by Clark *et al.* have shown that the LAC may have a higher association with pregnancy loss and adverse outcome than aCL.[43] The risk of fetal loss in women with circulating aPL has lately become controversial. Although some studies suggest that the presence of aCL or features of the antiphospholipid syndrome (APS) are associated with increased risk of pregnancy loss in women with SLE, others have not found this to be the case.[36,44–51] Questions have arisen regarding the association of aPL with early vs. late pregnancy loss; the significance of IgM and IgA isotypes of aCL,[52] and whether the presence of LAC or aCL in women with no history of thromboembolism or adverse pregnancy outcome, is sufficient indication for intervention.

p0290 Treatment for pregnant women with APS, whether primary or secondary, has also become somewhat controversial. Although initial studies supported the use of prednisone and aspirin to promote live birth in a woman with a history of pregnancy loss and aPL, a double-blind, randomized controlled trial failed to show a benefit beyond placebo.[53,54] Heparin and aspirin have become the accepted treatment for the prevention of pregnancy loss in women with APS,[55–60] although studies supporting the use of such therapy prior to 2000 contrast with those performed after that date where ASA alone was found to be at least as efficacious as heparin with ASA.[55–61]

The Neonate

s0120 In general, term births from women with SLE are at no greater risk of congenital anomalies than those born to mothers without SLE. The major exception is those babies born to mothers possessing anti-Ro (SSA) and/or anti-La (SSB) antibodies. Although abnormalities occur in only 1–2% of the neonates, the manifestations form collectively the neonatal lupus syndrome. Transient serologic abnormalities, skin lesions, and cardiac anomalies including congenital heart block characterize the syndrome,[62–64] which results from the transplacental passage of maternal IgG anti-Ro/La antibody and usually resolves by 8–9 months of age. The antibody may be found in up to 25–30% of women with SLE but may also be an isolated finding in the general population. Once a woman with anti-Ro/La antibodies has given birth to an infant with congenital heart block, the risk in a subsequent pregnancy is about 15%.[62]

p0270

The Management of SLE during Pregnancy

s0140 Ideally, lupus should be inactive when pregnancy is contemplated. Should the disease flare during pregnancy, treatment must be instituted immediately using the safest, most effective regimen. Prednisone has few adverse effects on the fetus and should be used if necessary. Other drugs with very good safety profiles in SLE include some non-steroidal anti-inflammatory drugs (NSAIDs, naproxen, ibuprofen), antimalarials, and azathioprine.

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Monitoring During Pregnancy

s0150 Both the obstetrician and internist should concurrently follow pregnant women with SLE. Efforts should be coordinated to avoid duplication of laboratory tests and inappropriate, overly liberal consultation with other medical specialties. Maternal clinical evaluation in addition to appropriate laboratory testing determines disease activity. If the patient's serology is concordant with disease activity, then rising anti-dsDNA antibody and falling complement levels will be the markers of a disease flare. Be aware that complement levels in pregnancy are typically elevated so a falling level should be noted, not just a low level. Validated measures of disease activity usually restricted to research protocols can be adapted for use in the clinic.[65,66]

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Labor and Delivery

s0160 The obstetrician should make the decision regarding the mode of delivery. Most patients with SLE deliver vaginally.

p0330

Corticosteroid supplementation should be administered at labor or prior to a cesarean section in women currently or recently using such medications. Of note, there is no rationale for prophylactically increasing the dose of corticosteroids to prevent a postpartum flare of the disease.[65]

Breastfeeding

p0380 The major concern in nursing the neonate is exposure to certain medications that may enter the breast milk. The underlying disease is not an issue in terms of the safety of the infant, but there is some conflicting evidence suggesting an association of disease exacerbation with hyperprolactinemia.[65–68] If the disease is active, caution must be exercised due to some medications with which the mother may be treated. Prednisone (up to 30–40 mg/day), hydroxychloroquine, and short acting non-steroidal agents (naproxen, ibuprofen) are considered compatible with breastfeeding.

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Contraception

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p0350 At one time combined oral contraceptives (estrogen-containing agents) were considered to be at least relatively contraindicated in women with SLE.[69–71] This may still be the case in those with aPL as there appears to be increased risk in those patients of thromboembolism.[72] However, in those lacking aPL the controversies have been limited to patients with some degree of disease activity. Two recent studies clarified this issue. Neither found any association between combined oral contraceptive use and exacerbation of SLE, providing the woman has stable disease.[73,74]

RHEUMATOID ARTHRITIS

s0190

p0360 Rheumatoid arthritis (RA) is a chronic, systemic, inflammatory disease targeting the joints. Its etiology is unknown but a peculiar host response to a viral infection continues to be hypothesized.[75] The disease appears to have no racial predilection and can be considered relatively common, affecting 1% of the population with a female to male ratio of 3:1. Although RA can affect any age group, most patients present between 20 and 60 years of age. The typical clinical picture is a symmetric, inflammatory polyarthritis mainly affecting small to medium-sized synovial joints. Early morning stiffness or gelling, and constitutional symptoms of fatigue and malaise, accompany active disease. The disease is characterized by exacerbations and remissions.[76] Extra-articular manifestations attest to the systemic nature of the disease process.

p0370 Laboratory findings include a normocytic, normochromic anemia as well as abnormalities in a number of acute phase reactants such as elevated platelet count and erythrocyte sedimentation rate (ESR), and hypergammaglobulinemia. Leukopenia is a manifestation of RA with hypersplenism, or Felty's syndrome. The classic autoantibody is an IgM anti-immunoglobulin or rheumatoid factor and is detected in the serum of 75–80% of patients with RA. The antinuclear factor (ANA) and several other autoantibodies are often found in Felty's syndrome. Synovial fluid is characterized by an inflammatory exudate with polymorphonuclear cells and lymphocytes. Radiographic changes range from soft tissue swelling to erosive destruction of the articular surfaces of joints.

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p0340 RA is diagnosed based upon the clinical and laboratory features outlined above. The ACR has revised the classification criteria for RA emphasizing the objective finding of synovitis for a minimum of 6 weeks, ruling out other conditions.[75,76]

Effect of Hormones and Pregnancy on RA

RA is a hormone-sensitive disease. Numerous studies have shown a relationship between the use of oral contraceptives (OC) and RA.[77–79] In some studies a protective effect of the higher estrogen containing OC was observed, particularly if used in younger age groups.[80] However, the use of OC does not affect the long-term outcome of RA should it develop.[78] Prolactin levels may exacerbate RA but breastfeeding for longer than 12 months may actually protect against development of the disease.[81] In contrast, breastfeeding for less than 12 months before the onset of RA is associated with an increased risk of developing RA and should it develop, the disease may be more aggressive.[82] Karlson *et al.* also reported that an early age at menarche and irregular menstrual cycles appear to be risk factors for the development of RA.[81]

p0400 There are very few diseases other than RA so dramatically affected by pregnancy. Remission of the disease occurs in almost 80% of pregnant women with RA, usually occurring in the first trimester but is also seen even into the second and third trimesters.[83,84] This observation was noted in 1938 when Hench described a marked improvement in rheumatoid disease in 33 of 34 pregnancies occurring in 20 women with RA.[85] Since that time, many others have described this tendency in RA.[84,86] In addition, there is evidence suggesting that pregnancy may exert a protective effect against the development of RA.[87,88]

p0410 In contrast to the remitting effects of pregnancy on RA, most patients flare 6 weeks to 6 months post partum.[89] In addition to confirming this observation, Spector and Da Silva noted an increased risk of developing RA during the postpartum period.[90] As mentioned above, some studies even suggest that a postpartum flare and the new onset of RA may be associated with breast-feeding. Barrett *et al.* found that women breastfeeding for the first time had increased disease activity 6 months postpartum.[82,91] It is hypothesized that high levels of the proinflammatory hormone prolactin plays a critical role in the increased incidence of postpartum flares of RA and the onset of new disease.

Not all patients experience the expected remission during pregnancy. Barrett *et al.* found that greater than 25% of women with RA continued to have significant disability during pregnancy. Surprisingly, in this prospective study, only 16% of 140 women with RA were in complete remission during pregnancy.[92]

Recent studies suggest that the effects of pregnancy on the clinical manifestations of RA may be more variable than originally accepted. There has been little success in predicting with any degree of certainty which women with RA will go into a remission during pregnancy. Factors that may be implicated in the variability of effect include disease duration, functional class, and rheumatoid factor positivity but none of these has been shown to have any predictive value. Although remission is expected by the end of the first trimester, there is still a small but significant number of women who show no improvement and indeed, require further intervention during pregnancy to control disease.

p0480

Possible Mechanisms of Remission during Pregnancy and Flares Post Partum

s0210

p0440 The remission-inducing effect of pregnancy on RA continues to be the subject of a great deal of creative research. Initially it was hypothesized that the increase in blood cortisol levels during pregnancy was responsible for the induction of remission in pregnant women with RA.[85,93] This theory has since been discredited because it has been shown that there is no correlation between the change in RA disease activity during pregnancy and plasma cortisol concentrations.[89] Furthermore, plasma cortisol levels normalize within 5 days post partum yet RA flares do not usually occur for another 4–6 weeks.

p0450 Non-hormonal plasma constituents may possibly play a role in remission induction in RA during pregnancy. Pregnancy zone proteins (PZP) have a suppressive effect on the inflammatory activity of polymorphonuclear leukocytes. It is interesting that the timing of amelioration of RA during pregnancy is associated with the rise of PZP levels. In addition, the lack of improvement in 20–25% of women with RA during a pregnancy may be due to the inability of these individuals to synthesize sufficient amounts of PZP.[84,94]

p0460 The expansion of T regulatory cells with suppressor activity and decreased B cell response in pregnancy (Th2 response) may counter the lack of suppressor activity seen in RA (Th1 response).[76,83,95] Therefore the amelioration of RA in pregnancy may be a downregulation of a Th1 response rather than an upregulation of a Th2 response.[95] Alternatively, the shift to Th2 cytokines during pregnancy may underlie the amelioration of RA and the shift back to a Th1 cytokine profile is responsible for the postpartum flare.[96–99]

p0470 An intriguing hypothesis has been proposed by Adams *et al.*[100] The hypothesis is based upon the recent observation that placental apoptotic syncytiotrophoblastic debris is extruded into the maternal circulation constituting the primary event. This debris contains intracellular fetal HLA Class II molecules. The second event is the uptake of this debris by immature maternal dendritic cells, which then present fetal HLA Class II peptides. The HLA molecules also present HLA self-peptides. In pregnancy, this process should induce immune tolerance of fetal antigens. In RA, the simultaneous presentation of fetal and self HLA peptides by tolerogenic dendritic cells during pregnancy may be the underlying mechanism of amelioration of disease activity. With the disappearance of the apoptotic syncytiotrophoblastic debris postpartum, the remission can no longer be sustained and there is a recurrence of rheumatoid disease activity. This novel hypothesis requires further investigation.

p0420

p0430

Yan *et al.* from this same group of investigators found that, in pregnancy, rheumatoid disease activity was correlated with maternal serum levels of fetal DNA.[101] As the fetal DNA levels increased throughout pregnancy, the disease was either markedly improved or remained in remission. Post partum, as fetal DNA levels decreased, disease activity returned.

Effect of RA on Pregnancy

s0220

p0490 Investigations regarding the effect of RA on pregnancy, as opposed to pregnancy on RA, are less well established. Indeed, there is little adverse disease effect on pregnancy with most issues being related to specific medications. Kaplan and Diamond noted that RA appears to have no significant impact on the patient's ability to have a normal pregnancy, delivery, and infant.[102] Nelson *et al.* found no increase in infertility in patients with RA, although there was diminished fecundability (the probability of conceiving within one menstrual cycle).[103] In a prospective case–control study this group also observed no increase in adverse pregnancy outcomes in women who later developed RA.[104] Notwithstanding these observations, women with an active inflammatory systemic disease often have menstrual irregularities with interruption of the pituitary–ovarian axis. This might lead to anovulation with subsequent subfertility. Upon establishing disease control these endocrine abnormalities reverse, with normalization of the menstrual cycle accompanied by ovulation and return of fertility.

Management of RA during Pregnancy

s0230

p0500 The therapeutic objective in both the pregnant and non-pregnant RA patient is to control inflammation, leading to reduction of pain, restoration of function, and prevention of deformity and damage. This is accomplished using a combination of drug therapy, patient education, physiotherapy, and occupational therapy.

p0510 Ideally the patient should defer pregnancy until she is well controlled on a treatment regimen deemed safe in pregnancy. A pre-pregnancy consultation will assist in this assessment allowing the woman to initiate a pregnancy electively with

a safe management plan in place. In RA, the major issues when contemplating pregnancy are mostly related to medications. Non-steroidal agents can be used in pregnancy with certain caveats (see below). Naproxen and ibuprofen have a good safety profile in pregnancy. Corticosteroids are not contraindicated but pre-pregnancy counseling for the patient is necessary regarding a moderately increased risk of orofacial cleft in the fetus.[105] Antimalarial agents such as hydroxychloroquine have become widely accepted in the treatment of the pregnant woman with RA.[106–111] Sulfasalazine can usually be continued during pregnancy with little if any risk to the mother and fetus.[107,112,113] It is worthwhile noting that males on sulfasalazine often manifest oligospermia, reversible upon discontinuation of the drug. Immunosuppressive agents such as azathioprine have shown a good safety track record in pregnancy whereas methotrexate should be discontinued 3 months prior to conception.[107] Even in males, it is recommended that methotrexate be discontinued 3 months prior to conception.[113] The question regarding tumor necrosis factor alpha (TNF-α) inhibitors has become very controversial recently[114] and at present, the use of such agents including etanercept and infliximab must be viewed as relatively contraindicated during a pregnancy.

Because most women with RA experience remission or at least a significant improvement in disease activity during pregnancy, medications can often be discontinued. For those with active disease during pregnancy, treatment must be directed at controlling inflammation while balancing the risk to the fetus with the benefits to the mother's health. In those women with active disease during pregnancy, the use of prednisone to control moderate to severe disease activity may be unavoidable.

Postpartum flare requires aggressive and timely treatment. If the mother is nursing, the drugs of choice for disease control are naproxen, ibuprofen, and prednisone. Antimalarials and sulfasalazine are also deemed safe for breastfeeding. The safety of TNF-α inhibitors for nursing mothers has not been established. A review of currently available data suggests that etanercept should be avoided during pregnancy and breastfeeding.[115] One case report found clinically significant levels of infliximab in a 6-week-old, breastfed newborn. As the investigators were unable to demonstrate infliximab in the breast milk, they concluded that its presence was the result of transplacental transfer and cautioned against maternal use during pregnancy as both short- and long-term effects on the infant as the result of exposure in utero are unknown.[116]

General therapeutic measures such as appropriate bedrest, physiotherapy, occupational therapy, and nutrition remain as mainstays in the treatment of active RA.

Issues Surrounding Labor and Delivery

Although there are few issues surrounding labor and delivery in women with RA, the usual issues with respect to surgery remain, particularly if rheumatoid disease involves the cervical spine with atlanto-axial subluxation and its attendant anesthetic risks. Recent population-based studies noted an increase risk of cesarean section, prematurity, and longer hospitalizations at birth among infants born to mothers with RA. These problems may actually be due to disease manifestations or maternal drug therapy.[117,118] There is a growing trend, especially in European hospitals, to establish practice guidelines with respect to the management of pregnant women with RA.[119]

Family Planning

Although there is no evidence indicating that RA adversely affects fertility or the ability to carry a pregnancy, counseling regarding medication safety during pregnancy remains the most significant issue when planning a pregnancy in a couple with RA. There are adverse effects on fertility among anti-inflammatory agents and certain immunosuppressants in both men and women.[120] Pregnancy itself is not harmful to the potential mother with RA or her infant but she must be cognizant of the emotional and physical stress associated in caring for a newborn.

SERONEGATIVE SPONDYLOARTHROPATHIES

This group of diseases is distinguished from RA by the absence of rheumatoid factor, or seronegativity. Among the diseases included in this category are ankylosing spondylitis (AS), reactive arthritis (formerly Reiter's syndrome), psoriatic arthritis (PsA), and arthritis associated with inflammatory bowel disease or enteropathic arthritis.[121] The common articular features of this group of disorders are sacroiliitis, spondylitis, seronegative polyarthritis, and dactylitis. It is intriguing to note the strong familial aggregation of these disorders both within each entity and among all the entities in the group. The finding of a strong association with HLA B27 supports the interrelationship of these diseases.

The diagnosis of a seronegative arthropathy is based upon clinical features including both articular and extra-articular, and the radiologic findings of sacroiliitis and spondylitis. Other than a negative rheumatoid factor, the laboratory findings are not very helpful: the ESR is usually normal. HLA typing may be helpful in dealing with a diagnostic challenge.

Other than PsA, seronegative disorders are more common in men than women, which distinguishes them from most other rheumatic diseases. The diagnosis of AS in a woman is often missed due to the milder nature of the disease process compared to that seen in men.[122]

Spondyloarthropathies and Pregnancy

There are few studies on the interaction of seronegative diseases and pregnancy. In his report of 14 patients with AS,

Hart concluded there was no change in the progress of the disease and childbirth had little or no effect on its course,[123] Others have observed that pregnancy has no ameliorating effect on AS and there is no adverse effect of the disease on the pregnancy.[124] The course of AS is independent of pregnancy, with each running a separate course. Flares during pregnancy occur but are likely the natural history of the disease rather than a relationship to pregnancy. In contrast, the majority of pregnant patients with PsA show an improvement in both skin and joint disease[125,126] and like RA, remission during pregnancy in PsA is relatively common (up to 70% of patients), and there is frequently a postpartum flare.

p0610 Treatment of AS as well as other seronegative diseases may involve the use of non-steroidal agents such as naproxen or ibuprofen. These agents can be used in pregnancy safely until week 32 when they must be discontinued. Corticosteroids may also be used and can be continued throughout pregnancy. Physical therapy is particularly helpful in treating sacroiliitis and spondylitis. In the pregnant woman, postural and breathing exercises may be especially important.

s0280 SCLERODERMA (PROGRESSIVE SYSTEMIC SCLEROSIS)

p0620 Progressive systemic sclerosis (PSS) is a relatively uncommon disease characterized by fibrosis and a vasculopathy in skin, joints, and internal organs.[125,127] The incidence of the disease is only 2–12 cases per million population annually, with a global distribution. Women are affected 3–4 times more than men, with most patients presenting between 30 and 50 years of age. The main pathologic lesion is vascular with a concentric proliferation of the intima and fibrosis of the adventitia of small arteries and arterioles.[127]

p0630 The most common clinical feature of PSS is Raynaud's phenomenon, which may predate other features by years. Skin and joint involvement is common as is dysphagia due to esophageal dysmotility. Renal involvement is the leading cause of death in PSS, typically manifested as the sudden onset of malignant hypertension. Poor prognostic signs in this disease include the presence of renal disease and pulmonary hypertension.[128]

p0640 Laboratory features of PSS are anemia due to chronic illness, microangiopathic hemolysis in cases of rapidly progressive renal failure, an elevated ESR, hypergammaglobulinemia, and a positive ANA. The diagnosis of PSS, however, is based on clinical features.[129]

s0290 Effect of Scleroderma on Pregnancy

p0650 There are few reports on scleroderma and pregnancy probably because it is such a rare disease, and it usually presents in postmenopausal women. Although there was some concern regarding an increase in infertility in women with PSS, Steen and Medsger were unable to confirm this when compared to controls.[130–132] An increased incidence of spontaneous abortions in women with PSS was observed in a case–control study, which included healthy controls.[132] Fourteen percent of pregnancies resulted in fetal demise usually in the first trimester and in women destined to develop PSS.[133] In addition, patients with PSS have higher rates of perinatal death and a greater frequency of small for gestational age neonates compared with a general population. However, even in women with severe PSS, successful pregnancies can occur if the complications of the disease are adequately treated in a timely fashion.[134,135]

Effect of Pregnancy on Scleroderma s0300

p0660 Early literature reported patients with diffuse PSS having an exacerbation of their disease during pregnancy to the point of maternal death.[136,137] However, in a later study using a questionnaire, authors reported that maternal complications were no more frequent than patients with RA or in normal controls.[131] A prospective study of 67 pregnancies demonstrated that in 61% symptoms remained stable, 20% of pregnancies improved, and the remainder noted worsening.[133]

p0670 If PSS progresses rapidly with cardiac or renal involvement, the effect of pregnancy may aggravate matters significantly.[137–141] Esophagitis is often exacerbated by PSS. Raynaud's phenomenon on the other hand usually improves during pregnancy due the peripheral vasodilation characteristic of pregnancy.[138]

p0680 Many patients do well during pregnancy, but for some, disease exacerbation may be precipitated by pregnancy. Pregnant women with PSS must be monitored closely by the internist/rheumatologist and obstetrician, with particular attention paid to blood pressure and renal function.

Treatment of Scleroderma s0310

p0690 There is no specific treatment for scleroderma. Treatment modalities are directed towards suppression of the microvascular abnormalities and the process underlying the progressive fibrosis characteristic of this condition.[125] To date, there are no pharmacologic or biologic agents that have demonstrated significant efficacy to warrant their recommendation in this condition. Management of scleroderma must be supportive and attempt to be preventative of disease progression whether the patient is pregnant or not. Supportive measures may include patient education, avoidance of exposure to cold and compliance with a physiotherapy program to preserve hand function and thereby minimize contractures.

p0700 Skin treatment in pregnancy is aimed at symptomatic relief of pruritis and the prevention of digital ulcers due to Raynaud's phenomenon. The usual treatment of Raynaud's phenomenon with calcium channel blocking agents such as nifedipine can also be used in pregnancy. In most pregnant women with Raynaud's phenomenon, the problem is

usually ameliorated by the generalized vasodilatation associated with pregnancy.

Systemic treatment of organ involvement is often necessary with medications selected to be safe in pregnancy. Antihypertensive agents such as alpha-methyldopa and calcium channel antagonists such as nifedipine are considered compatible with pregnancy and may be used to treat hypertension. If renal failure is present, dialysis may be required. In the case of severe cardiac, pulmonary or renal disease, termination of the pregnancy is recommended. Should myositis occur, corticosteroids are useful. Esophageal dysmotility leading to gastric reflux is common in scleroderma. It is a common problem in general in pregnancy and may be quite disabling when the woman also has scleroderma. Treatment with antacids, H2-blocking agents, and metoclopramide are often effective and may be used in pregnancy. The most effective therapy is proton pump inhibition. A multicenter prospective controlled study concluded that proton pump inhibitors do not represent a major teratogenic risk,[142] but the recommendation is still to use such agents only when the potential benefits outweigh the risks (US Food and Drug Administration, FDA, Category C-'Risk cannot be ruled out", Table 53.2). The Teratogen Information System, TERIS, concludes that the doses required during pregnancy would be unlikely to pose a risk to the fetus.

p0720 Pre-delivery anesthesia consultation may be beneficial owing to the special challenges inherent in scleroderma. Certain physical limitations may be present due to contractures of the skin, hips, and extremities. Ideally, during delivery the environment should be kept warm, including the provision of intravenous fluids. The patient should wear thermal socks and the application of warm compresses should be used to minimize problems of Raynaud's phenomenon, which can occur during labor and delivery.[138] Postpartum management involves monitoring the patient's blood pressure, which may be a harbinger of a renal crisis.

p0730 A pregnant woman with scleroderma usually experiences a good outcome medically and obstetrically. Owing to the rarity of this condition and the dearth of published literature concerning pregnancy in scleroderma, it is still not clear whether there is a higher frequency of pregnancy loss or adverse pregnancy outcome.[130,134,143] However, the adverse outcomes described in earlier literature do not seem to be as frequent today. Nonetheless, owing to the medical issues as well as the possibility of prematurity and small for gestational age neonate, women with scleroderma should attend a high-risk pregnancy unit and be managed by a perinatologist. Pre-pregnancy evaluation and planning as well as close medical and obstetrical monitoring to enable early and aggressive therapeutic intervention should promote a higher probability of a successful pregnancy outcome for both mother and neonate. p0710

t0020 **TABLE 53.2** FDA classification of drugs in pregnancy

Class	
A	Controlled studies show no risk
B	No evidence of risk in humans
C	Risk cannot be ruled out
D	Positive evidence of risk
X	Contraindicated in pregnancy

PHARMACOLOGIC TREATMENT OF RHEUMATIC DISEASE AND REPRODUCTION s0320

The assessment of any pregnant patient with rheumatic disease or those contemplating pregnancy must include consideration of current medications and, in some cases, previous exposure to specific pharmacologic agents. Although most drugs may be safe while the man and woman are attempting to conceive, some must be discontinued during pregnancy and yet others require discontinuation some time prior to conception. p0740

Non-steroidal Anti-inflammatory Drugs (NSAIDs) s0330

NSAIDs are commonly used in the treatment of the arthritis manifested in many rheumatic diseases. Many are now available over the counter, making it of paramount importance that the obstetrician and internist be aware of all medications consumed by the patient whether prescribed or self-administered. p0750

Most NSAIDs are safe to use for couples attempting to conceive. However, for women, some agents can inhibit follicular rupture, preventing the release of the oocyte and thereby contributing to subfertility.[144,145] This is not a common phenomenon, occurring in about 10% of women taking such agents (personal observations). In addition, NSAIDs may inhibit the motility of the fallopian tubes and by extension, the passage of the oocyte down the tubes.[146,147] Although these issues might arise with the use of any NSAID due to the inhibition of cyclooxygenase, the most widely studied agent is indomethacin.[148,149] p0760

During pregnancy, naproxen and ibuprofen are the two most commonly used NSAIDs. Indeed, there is too little in the literature regarding any other drug in this class other than indomethacin to have any knowledge regarding their safety profiles. When considering naproxen and ibuprofen in pregnancy, the fetal risk category is B but is reclassified as C when used in high doses. Peripartum there is concern with respect to the neonate regarding intracranial hemorrhage, premature closure of the ductus arteriosus, and impaired renal function leading to a decrease in amniotic fluid volume. The patient should be informed that NSAIDs will start being tapered by week 25 and completely discontinued by week 32 at the latest (6–8 weeks prior to the expected date of delivery). p0770

A population-based study in Denmark indicated that there might be some association with the use of NSAIDs and early pregnancy loss.[150] However, the study fails to indicate the reason for the use of the drugs or if there was an underlying disorder that might predispose to pregnancy loss. It is interesting to note that in women with RA, in whom the use of NSAIDs would be quite extensive, there does not appear to be any increased incidence of pregnancy loss. Clearly, further studies need to be undertaken to support or refute the Danish observation.

p0790 Aspirin (ASA) has become a commonly used drug in pregnancy.[151] Despite a continuing lack of consensus, it has been variously recommended as a pre-treatment for ovulation induction, for the promotion of implantation in in vitro fertilization cycles, and for the prevention of pregnancy loss.[152–154] It has been shown to provide moderate but consistent reductions in the relative risk of pre-eclampsia and preterm delivery.[155] ASA appears to be a safe agent to use in pregnancy with the exception of a reported increased incidence of gastroschisis in the offspring of women taking ASA in the first trimester.[156,157] Regardless of the very low frequency of this side effect (1/1000 compared to 1/10000 in the general population), patients should still be counseled regarding the potential association prior to initiating ASA therapy during pregnancy. ASA is also capable of prolonging labor and may cause an increase in antepartum and postpartum bleeding. It is actually for the latter reasons that it has an FDA classification of C/D when used in the third trimester. To avoid the issues surrounding labor and delivery, discontinuation of ASA 4 weeks prior to the expected date of delivery should be implemented.

p0800 The American Academy of Pediatrics (AAP) considers ASA, naproxen, and ibuprofen compatible with breastfeeding but as always, the lowest effective dose should be used.

s0340 ## Anticoagulants

p0810 The patient with APS and previous thrombosis should receive anticoagulation treatment throughout their pregnancy and during the postpartum period.[158] As discussed earlier, data supporting the use of heparin treatment in women with pregnancy loss and APL with no history of thrombosis are inconclusive and evidence-based standards of care both during the peripartum and postpartum periods are lacking.[159] Once the decision to use heparin has been made, low-molecular weight heparin (LMWH) is perceived to be more desirable than unfractionated heparin (UFH), despite a Cochrane Review recommending the use of unfractionated heparin[160] as LMWH carries less of a risk of osteoporosis and thrombocytopenia and can be administered once daily (161). Two small studies comparing UFH and LMWH did not show differences in efficacy but larger prospective data are needed.[162,163] LMWH treatment over the duration of a pregnancy can be associated with osteopenia and therefore calcium and vitamin D supplementation should be recommended during pregnancy.[164,165]

Antimalarial Agents p0780

Antimalarials are used extensively in both RA and SLE. p0820
The major side effect is retinal toxicity, which requires ophthalmologic monitoring every 6–12 months. The favored antimalarial is hydroxychloroquine (FDA Category C), which appears to have a lower incidence of retinal toxicity.

Numerous studies have attested to the safety of antimalarials in pregnancy.[106,166,167] All of the investigators have commented that the risk of a flare of disease far outweighs any risk of fetal toxicity. In a follow-up study of the children born to mothers on hydroxychloroquine during pregnancy, Klinger *et al.* found no evidence of retinal toxicity, prompting these authors to conclude that there appears to be little or no risk of ocular toxicity in children exposed to hydroxychloroquine in utero.[168] This observation has been confirmed by Motta *et al.*[169] p0830

The AAP has categorized hydroxychloroquine to be safe in the nursing mother. p0840

Corticosteroids s0360

Corticosteroids are commonly used in the treatment of most rheumatic diseases and are associated with a rapid or relatively rapid therapeutic response. In RA most patients achieve a response using low doses of prednisone (5–10 mg/day). In SLE, prednisone doses are usually higher, with minor organ disease requiring 10–40 mg/day and major organ manifestations treated with 40–80 mg/day. In all cases, the lowest effective dose of prednisone should be used. p0850

The use of prednisone in pregnancy is associated with few adverse side effects on the fetus. Maternal side effects are dose-related. The commonest side effects are hypertension and gestational diabetes mellitus. In a double-blind, randomized controlled trial, Laskin *et al.* observed an incidence of gestational diabetes mellitus of 15% and hypertension 13% in the prednisone-treated group compared to 5% in the placebo group for either condition.[54] The cushingoid side effects and osteopenia occur similarly to that seen in the non-pregnant state. p0860

Fetal side effects are few and uncommon. Orofacial clefting in the offspring of mothers treated with corticosteroids during the first trimester has been reported.[105,170] The results in these studies have been supported by the findings in a recent meta-analysis where the prevalence of orofacial clefting in prednisone-exposed infants was 1/400 compared to 1/800 in the general population.[171] In spite of the low risk of this potential side effect, any pregnant woman on corticosteroids should be counseled appropriately. p0870

Premature birth has been described in pregnant women treated with corticosteroids. In a randomized trial referred to above, Laskin *et al.* found premature births before 37 weeks gestation in 62% of the prednisone-treated group compared to 11% in the placebo group.[54] The neonates were all appropriate size for gestational age. Prednisone is classified as D p0880

when used in the first trimester. The physician must weight potential risks versus benefits when prescribing these agents.

p0890 The AAP considers prednisone to be compatible with breastfeeding. There appears to be minimal exposure with maternal doses at 30–40 mg/day.

s0370

Sulfasalazine

p0900 Sulfasalazine (SSZ) is used with reasonable success in RA, spondyloarthropathies, and inflammatory bowel disease. The drug appears to be safe in pregnancy with most of the evidence gathered from its use in Crohn's disease.[172–175] Although there are no reported issues with respect to female fertility, males treated with SSZ are often found to have low sperm counts and motility. The oligoasthenaspermia is reversible but requires at least 2 months avoidance of SSA.[172,176]

p0910 Owing to a report of bloody diarrhea in an infant breastfed by a woman on SSA, the AAP recommends caution when nursing on SSA.[177,178]

s0380

Azathioprine

p0920 Azathioprine (AZA) is used in many rheumatic diseases for its immunosuppressive properties and as a steroid-sparing agent. Among all immunosuppressive agents, AZA appears to be the safest in pregnancy. The placenta reportedly forms a relative barrier to AZA and its metabolites.[179] In a recent study of 189 women exposed to AZA compared to 230 controls not exposed to any teratogens during pregnancy, outcomes associated with AZA included increased rates of spontaneous abortions, intrauterine growth restriction (IUGR), and prematurity,[180] but no increase in the occurrence of major malformations. However, as larger studies are required to confirm these results, AZA continues to be a Class D drug.

p0930 With few data available regarding the safety of AZA in breastfeeding, the AAP has recommended that mothers avoiding nursing while being treated with this agent.

s0390

Methotrexate

p0940 Methotrexate (MTX), a drug used with good success in RA, is a folic acid antagonist. Use in pregnancy has been associated with spontaneous abortions due to embryotoxicity. The drug has definite association with numerous fetal anomalies as well as IUGR.[181–183] MTX is not to be used in pregnancy and has an FDA Category X rating.

p0950 Owing to MTX binding to tissues, it is recommended that it be discontinued at least 3 months prior to conception. A similar recommendation applies to men taking MTX but evidence is lacking to support such a recommendation.[182] However, until the situation is clarified, it is recommended that both men and women avoid pregnancy for at least 3 months after discontinuing MTX.[181–183]

p1010 MTX is only excreted in breast milk to a very small degree. However, it binds to neonatal tissues and therefore accumulates leading to toxicity.[183] The AAP categorizes MTX as contraindicated in nursing mothers.

Cyclophosphamide

s0400

p0970 Cyclophosphamide (CTX) is a cytotoxic, alkylating agent used in the treatment of severe major organ involvement in SLE and specific vasculitides. It has been categorized as a Class D drug by the FDA. CTX is embryotoxic and associated with many anomalies upon exposure in the first trimester. However, it does not appear to be associated with abnormalities if used in the second and third trimesters. Regardless, the drug should not be used in pregnancy unless there is a life-threatening problem and even then restricted to use late in the pregnancy. CTX is contraindicated in the nursing mother owing to the risk of neutropenia, immunosuppression, growth disturbances, and potential carcinogenesis in the neonate.[173,174,183]

Cyclosporine

s0410

p0980 Cyclosporine A (CSA, FDA Classification C) is used to treat certain manifestations of SLE renal disease. Most of the literature surrounding the use of CSA in pregnancy deals with renal transplantation. There appears to be little evidence that CSA crosses the placenta to any significance[184–186] and current data indicate that it is probably safe in pregnancy with no specific anomalies described.[187–189] Adverse pregnancy outcomes such as prematurity and IUGR likely have more to do with the underlying disease process than treatment with CSA.[190]

p0990 CSA is excreted in breast milk and is associated with immunosuppression, neutropenia, and growth disturbances in the neonate. The AAP categorizes CSA as contraindicated in the nursing mother.

Mycophenolate

s0420

p1000 Mycophenolate mofetil (MMF) is a purine biosynthesis inhibitor. Its use in pregnancy is accompanied by several congenital anomalies and spontaneous abortions.[187,191–193] These findings have been noted not only in animal studies but also in humans. In addition, a possible characteristic phenotype has been described.[192] Although MMF does not appear to impact male fertility, paternal exposure may be associated with congenital anomalies.[191] Recommendations in women regarding discontinuation of MMF prior to conception vary from 3 to 12 weeks. It would appear that avoidance of this drug by females at least 6 weeks prior to conceiving may be the most appropriate whereas for males, the recommendation is 12 weeks' avoidance. This is an FDA Class D drug and should be avoided in pregnancy.

p0960 Since MMF is excreted into breast milk, it should not be administered to nursing mothers.

s0430 **Leflunomide**

p1020 Leflunomide, a pyrimidine synthesis inhibitor, is used in the treatment of RA. The active metabolite has a half-life of 2 weeks. Owing to teratogenicity and embryotoxicity in animals when administered in human-equivalent doses, the drug is FDA Class X and is contraindicated in pregnancy. If pregnancy is contemplated, there is an elimination or washout protocol that should be undertaken by both men and women exposed to the drug. It may take up to 2 years after the last dosing before reaching undetectable levels. The elimination protocol is likely ineffective should the woman already be pregnant. It is therefore recommended that under such circumstances, the woman should be advised to terminate the pregnancy.

p1030 Evidence regarding the transfer of leflunomide into breast milk is insufficient, and until conclusive results are obtained, breastfeeding is therefore not recommended.[120]

s0440 **TNF-α Antagonists**

p1040 The two most commonly used anti-TNF-α agents are etanercept and infliximab, both of which are FDA Category B drugs and both of which are used in the treatment of RA, with infliximab being used most frequently in spondyloarthropathies. At present the data reported in the literature are very controversial.[187,194–199] While a review of 131 women directly exposed to infliximab during pregnancy found no increase in proportion of live births, miscarriages, or therapeutic terminations compared to the US general population,[199] a case report documented a possible causal relationship between maternal use of etanercept during pregnancy and the VATER or VACTERL complex of congenital anomalies (vertebral anomalies; anal atresia; cardiac defect; tracheoesophageal fistula; renal abnormalities; and limb abnormalities) occurring in the neonates.[196] Further studies into this association must be undertaken before a clear recommendation regarding the use of anti-TNF-α agents in pregnancy can be offered. At present, the common recommendation is to discontinue the agents once the woman is pregnant. Only in those cases where the mother's health is in jeopardy might anti-TNF-α agents be considered. However, all patients must be counseled regarding the potential fetal toxicity of these agents and the fact that very little is known about their use in pregnancy and lactation.

p1050 Other biologics such as rituximab, adalimumab, anakinra and abatacept, fall into a similar category as etanercept and infliximab except even less is known about their effects in pregnancy.[200,201]

There is insufficient information regarding these agents in the nursing mother, although one group of investigators, unable to demonstrate infliximab in the breast milk, concluded that its presence in a 6-week-old neonate was due to transplacental transfer. The maternal use of TNF-α antagonists should continue to be restricted until the short- and long-term effects on the infant resulting from exposure in utero and in breast milk have been more completely elucidated.[116]

CONCLUSION

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p1070 Management of pregnancy in women with rheumatic disease requires the physician to have a detailed knowledge of the patient's disease history, including her typical clinical manifestations, laboratory markers, and flare indicators. Ideally, a pregnancy should be planned with the patient to ensure conception occurs during a period of clinical quiescence after appropriate withdrawal of any fetotoxic agents. With improvements in disease management and perinatal monitoring, in addition to collaboration between rheumatologists and perinatologists, there is now a good prognosis for both mother and fetus for the majority of women with rheumatic disease.

References

1. Doria A, Iaccarino L, Arienti S *et al.* Th2 immune deviation induced by pregnancy: the two faces of autoimmune rheumatic diseases. *Reprod Toxicol* 2006;22:234–241.
2. Laskin CA. Pregnancy and the rheumatic diseases. In: GN Burrow, TP Duffy, JA Copel, eds. *Medical Complications During Pregnancy*, sixth ed. Philadelphia, PA: Elsevier Saunders; 2004:429–449.
3. Lahita RG, Chiorazzi N, Reeves WH, eds. *Textbook of the Autoimmune Diseases*. Philadelphia, PA: Lippincott Williams & Wilkins; 2000.
4. Roitt IM, Delves PJ. *Roitt's Essential Immunology*, tenth ed. London: Blackwell Science; 2001, 182.
5. Tan EM, Cohen AS, Tan EM *et al.* The 1982 revised criteria for the classification of systemic lupus erythematosus. *Arthritis Rheum* 1982;25:1271–1277.
6. Arnett FC. The genetics of human lupus. In: DJ Wallace, BH Hahn, eds. *Dubois' Lupus Erythematosus*, fifth ed. Baltimore, MD: Williams & Wilkins; 1997:77–117.
7. Gladman DD, Urowitz MB, Cole E, Ritchie S, Chang CH, Churg J. Kidney biopsy in SLE. 1: clinical-morphologic correlations. *Q J Med* 1989;73:1125–1133.
8. Fraga A, Mintz G, Orozco J. Sterility and fertility rates, fetal wastage and maternal morbidity in systemic lupus erythematosus. *J Rheumatol* 1974;1:283–288.
9. Sanchez-Guerrero J, Karlson EW, Liang MH, Hunter DJ, Speizer FE, Colditz GA. Past use of oral contraceptives and the risk of developing systemic lupus erythematosus. *Arthritis Rheum* 1997;40:804–808.
10. Huong DL, Wechsler B, Piette JC. Risks of ovulation induction therapy in systemic lupus erythematosus. *Br J Rheumatol* 1996;35:1184–1186.
11. Huong DL, Wechsler B, Vauthier-Brouzes D *et al.* Importance of planning ovulation in systemic lupus erythematosus and antiphospholipid syndrome: a single center retrospective study of 21 cases and 114 cycles. *Semin Arthritis Rheum* 2002;32:174–188.

p1060

12. Guballa N, Sammaritano L, Schwartzman S, Buyon J, Lockshin MD. Ovulation induction and in vitro fertilization in systemic lupus erythematosus and antiphospholipid syndrome. *Arthritis Rheum* 2002;43:550–556.
13. Ellis FA, Bereston ES. Lupus erythematosus associated with pregnancy and menopause. *AMA Arch Dermatol Syphilol* 1952;69:170–176.
14. Garsenstein M, Pollak VE, Kark RM. Systemic lupus erythematosus and pregnancy. *N Engl J Med* 1962;267:165–169.
15. Mund A, Simson J, Rothfield N. Effect of pregnancy on the course of systemic lupus erythematosus. *JAMA* 1963;183:917–920.
16. Zurier RB. Systemic lupus erythematosus and pregnancy. *Clin Rheum Dis* 1975;1:613–616.
17. R.R. Grigor, P.C. Shervington, G.R.V. Hughes, D.F. Hawkins, Outcome of pregnancy in systemic lupus erythematosus, Proc. R. Soc. Med. 70 99–100.
18. Tozman EC, Urowitz MB, Gladman DD. Systemic lupus erythematosus and pregnancy. *J Rheumatol* 1980;7:624–632.
19. Zulman MI, Talal N, Hoffman GS, Epstein WV. Problems associated with the management of pregnancies in patients with systemic lupus erythematosus. *J Rheumatol* 1980;7:37–49.
20. Lockshin MD, Reinitz E, Druzin ML, Murrman M, Estes D. Lupus pregnancy: case control study demonstrating absence of lupus exacerbations during and after pregnancy. *Am J Med* 1984;77:893–898.
21. Lockshin MD. Pregnancy does not cause systemic lupus erythematosus to worsen. *Arthritis Rheum* 1989;32:665–670.
22. Petri M. Hopkins lupus pregnancy center: 1987–1996. *Rheum Dis Clin North Am* 1997;23:1–13.
23. Ruiz-Irastortza G, Lima F, Alves J *et al.* Increased rate of lupus flare during pregnancy and the puerperium: a prospective study of 78 pregnancies. *Br J Rheumatol* 1996;35:133–138.
24. Cortes-Hernandez J, Ordi-Ros J, Paredes F, Casellas M, Castillo F, Vilardell-Tarres M. Clinical predictors of fetal and maternal outcome in systemic lupus erythematosus: a prospective stud of 103 pregnancies. *Rheumatology (Oxford)* 2002;41:643–650.
25. Urowitz MB, Gladman DD, Farewell VT, Stewart J, McDonald J. Lupus and pregnancy studies. *Arthritis Rheum* 1993;36:1392–1397.
26. Yasmeen S, Wilkins EE, Field NT, Sheikh RA, Gilbert WM. Pregnancy outcomes in women with systemic lupus erythematosus. *J Maternal Fetal Med* 2001;10:91–96.
27. Clark CA, Spitzer KA, Nadler JN, Laskin CA. Preterm deliveries in women with systemic lupus erythematosus. *J Rheumatol* 2003;30:2127–2132.
28. Jungers P, Dougados M, Pelissier C *et al.* Lupus nephropathy and pregnancy. *Arch Intern Med* 1982;142:771–776.
29. Huong DL, Wechsler B, Vauthier-Brouzes D *et al.* Pregnancy in past or present lupus nephritis: a study of 32 pregnancies from a single centre. *Ann Rheum Dis* 2001;60:599–604.
30. Bobrie G, Liote F, Houillier P *et al.* Pregnancy in lupus nephritis and related disorders. *Am J Kidney Dis* 1987;9:339–343.
31. Moroni G, Quaglini S, Banfi G *et al.* Pregnancy in lupus nephritis. *Am J Kidney Dis* 2002;40:713–720.
32. Tandon A, Ibaniz D, Gladman DD, Urowitz MB. The effect of pregnancy on lupus nephritis. *Arthritis Rheum* 2004;50:3941–3946.
33. Nossent HC, Swaak TJ. Systemic lupus erythematosus. VI. Analysis of the interrelationship with pregnancy. *J Rheumatol* 1990;17:771–776.
34. Morris WI. Pregnancy in rheumatoid arthritis and systemic lupus erythematosus. *Aust N Z J Obstet Gynecol* 1969;9:136–144.
35. Friedman EA, Rutherford JW. Pregnancy and lupus erythematosus. *Obstet Gynecol* 1956;8:601–610.
36. Lima F, Buchanan NMM, Kamashta MA, Kerslake S, Hughes GR. Obstetric outcome in systemic lupus erythematosus. *Semin Arthritis Rheum* 1995;25:184–192.
37. Clark CA, Spitzer KA, Laskin CA. Decrease in pregnancy loss rates in patients with systemic lupus erythematosus over a 40-year period. *J Rheumatol* 2005;32:1709–1712.
38. McNeill HP, Chesterman CN, Krilis SA. Immunology and clinical importance of antiphospholipid antibodies. *Adv Immunol* 1991;8:425–426.
39. Tseng CE, Buyon JP. Neonatal lupus syndrome. *Rheumatic Dis Clin North Am* 1997;23:31–54.
40. Elias M, Eldor A. Thromboembolism in patients with the "lupus" type circulating anticoagulant. *Arch Intern Med* 1984;144:510–515.
41. Mueh JR, Herbst KD, Rapaport SI. Thrombosis in patients with the lupus anticoagulant. *Ann Intern Med* 1980;92:156–159.
42. Lockshin MD, Druzin ML, Goei S *et al.* Antibody to cardiolipin as a predictor of fetal distress or death in pregnant patients with systemic lupus erythematosus. *N Engl J Med* 1985;313:152–156.
43. Clark-Soloninka CA, Spitzer KA, Nadler JN, Laskin CA. Evaluation of screening 590 plasma samples for the lupus anticoagulant using a panel of four tests. *Arthritis Rheum* 1998;41(Suppl.):S168.
44. Martinez-Rueda JO, Arce-Salinas CA, Kraus A, Alcocer-Varela J, Alarcon-Segovia D. Factors associated with fetal loss in severe systemic lupus erythematosus. *Lupus* 1996;5:113–119.
45. Hanly JG, Gladman DD, Rose TH, Laskin CA, Urowitz MB. Lupus pregnancy: a prospective study of placental changes. *Arthritis Rheum* 1998;31:358–366.
46. Abramowsky CR, Vegas ME, Swinehart G, Gyves MT. Decidual vasculopathy of the placenta in lupus erythematosus. *N Engl J Med* 1980;303:668–672.
47. Guzman E, Schulman H, Bracero L, Rochelson B, Farmakides G, Coury A. Uterine-umbilical artery Doppler velocimetry in pregnant women with systemic lupus erythematosus. *J Ultrasound Med* 1992;11:275–281.
48. Carreras LO, Defreyn G, Machin SJ *et al.* Arterial thrombosis, intrauterine death and "lupus" anticoagulant: Detection of immunoglobulin interfering with prostacyclin formation. *Lancet* 1981;1:244–246.
49. Geis W, Branch DW. Obstetric implications of antiphospholipid antibodies: pregnancy loss and other complications. *Clin Obstet Gynecol* 2001;44:2–10.
50. Out HJ, Bruinsem HW, Chrustuaebs GCML *et al.* A prospective, controlled multicenter study on the obstetric risks of pregnant women with antiphospholipid antibodies. *Am J Obstet Gynecol* 1992;167:26–32.
51. Vinatier D, Dufour P, Cosson M, Houpeau JL. Antiphospholipid syndrome and recurrent miscarriages. *Eur J Obstet Gynecol Reprod Biol* 2001;96:37–50.
52. Soloninka CA, Laskin CA, Wither J, Wong D, Bombardier C, Raboud J. Clinical utility and specificity of anticardiolipin antibodies. *J Rheumatol* 1991;18:1849–1855.

53. Lubbe WFF, Butler WS, Palmer SJ, Liggins GC. Fetal survival after prednisone suppression of maternal lupus anticoagulant. *Lancet* 1983;2:1361–1363.
54. Laskin CA, Bombardier C, Hannah M *et al.* Prednisone and aspirin in women with autoantibodies and unexplained recurrent fetal loss. *N Engl J Med* 1997;337:148–153.
55. Rai R, Cohen H, Dave M, Regan L. Randomized controlled trial of aspirin and aspirin plus heparin in pregnant women with recurrent miscarriage associated with phospholipids antibodies (or antiphospholipid antibodies). *Br Med J* 1997;314:253–57.
56. Huong DL, Wechsler B, Bletry O, Vauthier-Brouzes D, Lefebvre G, Piette JC. A study of 75 pregnancies in patients with antiphospholipid syndrome. *J Rheumatol* 2001;28:2025–30.
57. Farquharson RG, Quenby S, Greaves M. Antiphospholipid syndrome in pregnancy: a randomized, controlled trial of treatment. *Obstet Gynecol* 2002;100:408–13.
58. Shehata HA, Nelson-Piercy C, Khamashta MA. Management of pregnancy in antiphospholipid syndrome. *Rheum Dis Clin North Am* 2001;27:643–59.
59. Laskin CA, Spitzer KA, Clark CA *et al.* Low molecular weight heparin and aspirin for recurrent pregnancy loss: results from the randomized, controlled HepASA Trial. *J Rheumatol* 2009;36:279–87.
60. Bresnihan B, Grigor RR, Oliver M *et al.* Immunological mechanism for spontaneous abortion in systemic lupus erythematosus. *Lancet* 1997;2:1205–7.
61. Empson M, Lassere M, Craig JC, Scott JR. Recurrent pregnancy loss with antiphospholipid antibody: a systematic review of therapeutic trials. *Obstet Gynecol* 2002;100:135–44.
62. Escobar MC, Gomez-Puerta JA, Albert D, Ferrer Q, Girona J. Recurrent congenital heart block in neonatal lupus. *Clin Rheumatol* 2006;26:1161–63.
63. Brucato A, Doria A, Frassi M *et al.* Pregnancy outcome in 100 women with autoimmune diseases and anti-Ro/SSA antibodies: a prospective controlled study. *Lupus* 2002;11:716–21.
64. Lee LA, Harmon CE, Huff C, Norris DA, Weston WL. The demonstration of SSA/Ro antigen in human fetal tissues and in neonatal and adult skin. *J Invest Dermatol* 1985;85:143–46.
65. Ruiz-Irastorza G, Lima F, Alvers J *et al.* Increaed rate of lupus flare during pregnancy and the puerperium: a prospective study of 78 pregnancies. *Br J Rheumatol* 1996;35:133–38.
66. Urowitz MB, Gladman DD. Measures of disease activity and damage in SLE. *Baillière's Clin Rheumatol* 1998;12:405–13.
67. Mok CC, Wong RW, Lau CS. Exacerbation of systemic lupus erythematosus by breast feeding. *Lupus* 1998;7:569–70.
68. Ostensen M. Sex hormones and pregnancy in rheumatoid arthritis and systemic lupus erythematosus. *Ann N Y Acad Sci* 1999;876:131–43.
69. Cooper GS, Dooley MA, Treadwell EL, St Clair EW, Gilkeson GS. Hormonal and reproductive risk factors for development of systemic lupus erythematosus: results of a population-based, case-control study. *Arthritis Rheum* 2002;46:1830–39.
70. Gill D. Rheumatic complaints of women using antiovulatory drugs. *J Chron Dis* 1968;21:435–44.
71. Dubois EL, Strain L, Ehn M, Bernstein G, Friou GJ. LE cells after oral contraceptives. *Lancet* 1968;2:679.
72. Chapel TA, Burns RE. Oral contraceptives and exacerbation of lupus erythematosus. *Am J Obstet Gynecol* 1971;110:366–69.
73. Petri M, Kim MY, Kalunian KC *et al.* Combined oral contraceptives in women with systemic lupus erythematosus. *N Engl J Med* 2005;353:2550–58.
74. Sanchez-Guerrero J, Uribe AG, Jimenez-Santana L *et al.* A trial of contraceptive methods in women with systemic lupus erythematosus. *N Engl J Med* 2005;353:2539–49.
75. Firestein G. Etiology and pathogenesis of rheumatoid arthritis. In: WN Kelley, ED Harris, S Ruddy, CB Sledge, eds. Textbook of Rheumatology, fifth ed.vol. I. Philadelphia, PA: WB Saunders; 1997.
76. Harris ED Jr. Clinical features of rheumatoid arthritis. In: WN Kelley, ED Harris, S Ruddy, CB Sledge, eds. Textbook of Rheumatology, fifth ed.vol. I. Philadelphia, PA: WB Saunders; 1997.
77. Merlino LA, Cerhan JR, Cirswell LA, Mikuls TR, Saag KG. Estrogen and other female reproductive risk factors are not strongly associated with the development of rheumatoid arthritis in elderly women. *Semin Arthritis Rheum* 2003;33:72–82.
78. Drossaers-Bakker KW, Zwinderman AH, van Zeben D, Breedveld FC, Hazes JM. Pregnancy and oral contraceptive use do not significantly influence outcome in long term rheumatoid arthritis. *Ann Rheum Dis* 2002;61:405–8.
79. Bhatia SS, Majkam DS, Kitelson JM, Norris JM *et al.* Rheumatoid factor seropositivity is inversely associated with oral contraceptive use in women without rheumatoid arthritis. *Ann Rheum Dis* 2007;66:267–69.
80. Doran MF, Crowson CS, O'Fallon WM, Gabriel SE. The effect or oral contraceptives and estrogen replacement therapy on the risk of rheumatoid arthritis: a population based study. *J Rheumatol* 2004;31:207–13.
81. Karlson EW, Mandl LA, Hankinson SE, Grodstein F. Do breast-feeding and other reproductive factors influence future risk of rheumatoid arthritis? Results from the Nurses' Health Study. *Arthritis Rheum* 2004;50:3458–67.
82. Jorgensen C, Piot MC, Bologna C, Sany J. Oral contraception, parity, breast feeding, and severity of rheumatoid arthritis. *Ann Rheum Dis* 1996;55:94–98.
83. Ostensen M, Villiger PM. Immnunology of pregnancy-pregnancy as a remission inducing agent in rheumatoid arthiritis. *Transplant Immunol* 2002;9:155–60.
84. Persellin RH. The effect of pregnancy on rheumatoid arthritis. *Bull Rheum Dis* 1976-1977;27:922–27.
85. Hench PS. The amelioration effect of pregnancy on chronic atrophic (infectious) rheumatoid arthritis, fibrositis and intermittent hydrarthrosis. *Proc Mayo Clinic* 1938;13:161.
86. Bulmash JM. Rheumatoid arthritis and pregnancy. *Obstet Gynecol Ann* 1979;8:223–76.
87. Hazes JMW, Dukmans BAC, Vandenbroucke JP, de Vries RR, Cats A. Pregnancy and the risk of developing rheumatoid arthritis. *Arthritis Rheum* 1990;33:1770–75.
88. Silman A, Kay A, Brennan P. Timing of pregnancy in relation to the onset of rheumatoid arthritis. *Arthritis Rheum* 1992;35:152–55.
89. Nelson JL, Ostensen M. Pregnancy and rheumatoid arthritis. *Rheum Dis Clin North Am* 1997;23:195–221.
90. Spector TD, Da Silva JAP. Pregnancy and rheumatoid arthritis: an overview. *Am J Reprod Immunol* 1992;28:222–25.
91. Barret JH, Brennan P, Fiddler M, Silman A. Breast-feeding and postpartum relapse in women with rheumatoid and inflammatory arthritis. *Arthritis Rheum* 2000;43:1010–15.

92. Barrett JH, Brennan P, Fiddler M, Silman AJ. Does rheumatoid arthritis remit during pregnancy and relapse postpartum? Results from a nationwide study in the United Kingdom performed prospectively from late pregnancy. *Arthritis Rheum* 2000;42:1219–27.
93. Plotz CM, Goldenberg A. Rheumatoid arthritis. In: JJ Ravinsky, AF Gutman, eds. *Medical, Surgical and Gynecological Complications of Pregnancy*, second ed. Baltimore, MD: Williams & Wilkins; 1965.
94. Neely NT, Persellin RH. Activity of rheumatoid arthritis during pregnancy. *Texas Med* 1977;73:59–63.
95. Ostensen M, Villiger PM. The remission of rheumatoid arthritis during pregnancy. *Semin Immunopathol* 2007;29:185–91.
96. Shimoaka Y, Hidaka Y, Tada H *et al.* Changes in cytokine production during and after normal pregnancy. *Am J Reprod Immunol* 2000;44:143–47.
97. Elenkov IJ, Wilder RL, Bakalov VK *et al.* IL-12, TNF-alpha, and hormonal changes during late pregnancy and early postpartum: implications for autoimmune disease activity during these times. *J Clin Endocrinol Metabol* 2001; 86:4933–38.
98. Weetman AP. The immunology of pregnancy. *Thyroid 1989* 1999;9:643–46.
99. Van Roon JA, Bijlsma JW, Lafeber FP. Suppression of inflammation and joint destruction in rheumatoid arthritis may require a concerted action of Th2 cytokines. *Curr Opin Invest Drugs* 2002;3:1011–16.
100. Adams KM, Yan Z, Stevens AM, Nelson JL. The changing maternal "self" hypothesis: a mechanism for maternal tolerance of the fetus. *Placenta* 2007;28:378–82.
101. Yan A, Lambet NC, Ostensen M, Adams KM, Guthrie KA, Nelson JL. Prospective study of fetal DNA in serum and disease activity during pregnancy in women with inflammatory arthritis. *Arthritis Rheum* 2006;54:2069–73.
102. Kaplan D, Diamond H. Rheumatoid arthritis and pregnancy. *Clin Obstet Gynecol* 1965;8:286–303.
103. Nelson JL, Koepsell RD, Dugowson CE, Voigt LF, Daling JR, Hansen JA. Fecundity before disease onset in women with rheumatoid arthritis. *Arthritis Rheum* 1993;36:7–14.
104. Nelson JL, Voigt LF, Koepsell TD, Dugowson CE, Daling JR. Pregnancy outcome in women with rheumatoid arthritis before disease onset. *J Rheumatology* 1992;19:18–21.
105. Carmichael SL, Shaw GM, Ma Cand the National Birth Defects Prevention Study *et al.* Maternal corticosteroid use and orofacial cleft. *Am J Obstet Gynecol* 2007;197:el-585–87.
106. Al-Herz A, Schulzer M, Esdaile JM. Survey of antimalarial use in lupus pregnancy and lactation. *J Rheumatol* 2002;29:700–6.
107. Ostensen M, Ramsey-Goldman R. Treatment of inflammatory rheumatic disorders in pregnancy: what are the safest treatment options?. *Drug Safety* 1998;19:389–410.
108. Costedoat-Chalumeau N, Amoura Z, Duhaut P *et al.* Safety of hydroxychloroquine in pregnant patients with connective tissue diseases: a study of one hundred thirty-three cases compared with a control group. *Arthritis Rheum* 2003;48:3207–11.
109. Levy RA, Vilela VS, Cataldo MJ *et al.* Hydroxychloroquine (HCQ) in lupus pregnancy: double blind and placebo controlled study. *Lupus* 2001;10:401–4.
110. Borden MB, Parke AL. Antimalarial drugs in systemic lupus erythematosus: use in pregnancy. *Drug Safety* 2001;24:1055–63.
111. Parke A, West B. Hydroxychloroquine in pregnant patients with systemic lupus erythematosus. *J Rheumatol* 1996;23:1715–18.
112. Rains CP, Noble S, Faulds D. Sulfasalazine. A review of its pharmacological properties and therapeutic efficacy in the treatment of rheumatoid arthritis. *Drugs* 1995;50:137–56.
113. Janseen NM, Genta MS. The effects of immunosuppressive and anti-inflammatory medications on fertility, pregnancy and lactation. *Arch Intern Med* 2000;160:610–19.
114. Carter JD, Ladhani A, Ricca L, Valeriano J, Vasey FB. A safety assessment of TNF antagonists during pregnancy: a review of the FDA database. *J Rheumatol* 2009;36:635–41.
115. Romero-Mate A, Garcia-Domoso C, Cordoba-Guijarro S. Efficacy and safety of etanercept in psoriasis/psoriatic arthritis: an updated review. *Am J Clin Dermatol* 2007;8:143–53.
116. E.A. Vasiliauskas, J.A. Church, N. Silverman, M. Barry, S.R. Targan, M.C. Dubinsky, Case report: evidence for transplacental transfer of maternally administered infliximab to the newborn, Clin. Gastroenterol. Hepatol. 4 1255–1258.
117. Reed SD, Voltan TA, Svec MA. Pregnancy outcomes in women with rheumatoid arthritis in Washington State. *Maternal Child Health J* 2006;10:361–66.
118. Chakravarty EF, Nelson L, Krishnan E. Obstetric hospitalizations in the United States for women with systemic lupus erythematosus and rheumatoid arthritis. *Arthritis Rheum* 2006;54:899–907.
119. Vroom F, van der Laar MA, van Roon EN, Brouwers JR, de Jong-van den Berg LT. Treatment of pregnancy and non-pregnant rheumatic patients: a survey among Dutch rheumatologists. *J Clin Pharm Ther* 2009;833:39–44.
120. Ostensen M. Antirheumatic therapy and reproduction. The influence on fertility, pregnancy and breast feeding. *Z Rheumatol* 2006;65:222–24.
121. Benjamin R, Parham P. HLA-B27 and disease: a consequence of inadvertent antigen presentation. *Rheum Dis Clin North Am* 1992;18:11–21.
122. Moll JMH. The place of psoriatic arthritis in the spondarthritides. *Baillière's Clin Rheumatol* 1994;8:395–417.
123. Hart FD. Discussion on medical diseases in pregnancy. *Proc R Soc Med* 1959;52:767–74.
124. Ostensen M, Husby G. Ankylosing spondylitis and pregnancy. *Rheum Dis Clin North Am* 1989;15:241–54.
125. Ostensen M. The effect of pregnancy on ankylosing spondylitis, psoriatic arthritis, and juvenile rheumatoid arthritis. *Am J Reprod Immunol* 1992;28:235–37.
126. Gladman DD. Natural history of psoriatic arthritis. *Baillière's Clin Rheumatol* 1994;8:379–94.
127. Seibold JR. Scleroderma. In: WN Kelley, ED Harris, S Ruddy, CB Sledge, eds. Textbook of Rheumatology, fifth ed.Vol. I. Philadelphia, PA: WB Saunders; 1997.
128. Lee P, Langevitz P, Alderdice CA *et al.* Mortality in systemic sclerosis (scleroderma). *Q J Med* 1992;82:139–48.
129. Masi A. for Subcommittee for Scleroderma Criteria of the American Rheumatism Association Diagnostic and Therapeutic Criteria Committee. Preliminary criteria for the classification of systemic sclerosis (scleroderma). *Arthritis Rheum* 1980;23:581–90.

130. Silman AJ, Black C. Increased incidence of spontaneous abortion and infertility in women with scleroderma before disease onset: a controlled study. *Ann Rheum Dis* 1988;47:441–44.
131. Englert H, Brennan P, McNeil D, Black C, Silman AJ. Reproductive function prior to disease onset in women with scleroderma. *J Rheumatol* 1992;19:1575–79.
132. Steen VD, Medsger TA Jr. Fertility and pregnancy outcome in women with systemic sclerosis. *Arthritis Rheum* 1999;42:763–68.
133. Steen VD, Brodeur M, Conte C. Prospective pregnancy (PG) study in women with systemic sclerosis (Ssc). *Arthritis Rheum* 1996;39(Supp. 9):S151.
134. Giordano M, Valentini G, Lupoli S, Giordano A. Pregnancy and systemic sclerosis. *Arthritis Rheum* 1985;28:237–38.
135. Steen VD. Scleroderma and pregnancy. *Rheum Dis Clin North Am* 1997;23:133–47.
136. Sampaio-Barros PD, Samara AM, Marques Neto JF. Gynaecologic history in systemic sclerosis. *Clin Rheumatol* 2000;19:184–87.
137. Spiera H, Krakoff L, Fishbane-Mayer J. Successful pregnancy after scleroderma hypertensive renal crisis. *J Rheumatol* 1989;16:1587–98.
138. Avrech OM, Golanm A, Panskym M, Langerm R, Caspi E. Raynaud's phenomenon and peripheral gangrene complicating scleroderma in pregnancy – diagnosis and management. *Br J Obstet Gynaecol* 1992;99:850–51.
139. Black CM. Systemic sclerosis and pregnancy. *Baillière's Clin Rheumatol* 1990;4:105–24.
140. Maymon R, Fejgin M. Scleroderma in pregnancy. *Obstet Gynecol Surv* 1989;44:530–34.
141. Brown AN, Bolster MB. Scleroderma renal crisis in pregnancy associated with massive proteinuria. *Clin Exper Rheumatol* 2003;21:114–16.
142. Diav-Citrin O, Arnon J, Shechtman S *et al.* The safety of proton pump inhibitors in pregnancy: a multicentre prospective controlled study. *Aliment Pharmacol Ther* 2005;21:269–75.
143. Steen VD, Conte C, Day N, Ramsey-Goldman R, Medsger TA Jr. Pregnancy in women with systemic sclerosis. *Arthritis Rheum* 1989;32:151–57.
144. Stone S, Khamashta MA, Nelson-Piercy C. Nonsteroidal anti-inflammatory drugs and reversible female infertility: is there a link?. *Drug Safety* 2002;25:545–51.
145. Killick S, Elstein M. Pharmacologic production of luteinized unruptured follicles by prostaglandin synthetase inhibitors. *Fertil Steril* 1987;47:773–77.
146. Elder MG, Myatt L, Chaudhuri G. The role of prostaglandins in the spontaneous motility of the fallopian tube. *Fertil Steril* 1997;28:86–90.
147. Laszlo A, Nadasy GL, Monos E, Zsolnai B. Effect of pharmacological agents on the activity of the circular and longitudinal smooth muscle layers of human fallopian tube ampullar segments. *Acta Physiol Hungarica* 1988;72:123–33.
148. Sookvanichsilp N, Pulbutr P. Anti-implantation effects of indomethacin and celecoxib in rats. *Contraception* 2002;65:373–78.
149. Lim H, Paria BC, Das SK *et al.* Mulitple female reproductive failures in cyclooxygenase 2-deficient mice. *Cell* 1997;91:197–208.
150. Nielsen GL, Sorensen HT, Larsen H, Pedersen L. Risk of adverse birth outcome and miscarriage in pregnant users of non-steroidal anti-inflammatory drugs: population based observational study and case-control study. *Br Med J* 2001;322:266–70.
151. James AH, Brancazio LR, Price T. Aspirin and reproductive outcomes. *Obstet Gynecol Surv* 2008;63:49–57.
152. Bromer JG, Cetinkaya MB, Arici A. Pretreatments before the induction of ovulation in assisted reproduction technologies: evidence-based medicine in 2007. *Ann N Y Acad Sci* 2008;1127:31–40.
153. Frattarelli JL, McWilliams GD, Hill MJ, Miller KA, Scott RT Jr. Low-dose aspirin use does not improve in vitro fertilization outcomes in poor responders. *Fertil Steril* 2008;89:1113–17.
154. Jauniaux E, Farquharson RG, Christiansen OB, Exalto N. Evidence-based guidelines for the investigation and medical treatment of recurrent miscarriage. *Human Reprod* 2006;21:2216–22.
155. Askie LM, Duley L, Henderson-Smart DJ, Stewart LA and the PARIS Collaborative Group. Antiplatelet gents for prevention of pre-eclampsia: a meta-analysis of individual patient data. *Lancet* 2007;369:1791–98.
156. Kozer E, Nikrar S, Costei A, Boskovic R, Hulman I, Koren G. Aspirin consumption during the first trimester of pregnancy and congenital anomalies: a meta-analysis. *Am J Obstet Gynecol* 2002;187:1623–30.
157. Norgard B, Puho E, Czeizel AE, Skriver MV, Sorensen HT. Aspirin use during early pregnancy and the risk of congenital abnormalities: a population-based case–control study. *Am J Obstet Gynecol* 2005;192:922–23.
158. Riuz-Irastorza G, Khamashta MA. Management of thrombosis in antiphospholipid syndrome and systemic lupus erythematosus in pregnancy. *Ann N Y Acad Sci* 2005;1051:606–12.
159. Spitzer KA, Murphy D, Crowther M, Clark CA, Laskin CA. Postpartum management of women at increased risk of thrombosis – results of a Canadian pilot study. *J Rheumatol* 2006;33:2222–26.
160. Empson M, Lassere M, Craig J, Scott J. Prevention of recurrent miscarriage for women with antiphospholipid antibody or lupus anticoagulant. *Cochrane Database System Reviews* 2005;18, CDD002859.
161. Greer IA. Anticoagulants in pregnancy. *J Thromb Thrombolysis* 2006;21:57–65.
162. Stephenson MD, Ballem PJ, Tsang P. Treatment of antiphospholipid antibody syndrome (APS) in pregnancy: a randomized pilot comparing low molecular weight heparin to unfractionated heparin. *Can J Obstet Gynecol* 2004;26:729–34.
163. Noble LS, Kutteh WH, Lashey N, Frandklin RD, Herrad J. Antiphospholipid antibodies associated with recurrent pregnancy loss: prospective, multicenter, controlled pilot study comparing treatment with low molecular weight heparin vs unfractionated heparin. *Fertil Steril* 2005;83:684–90.
164. Ruiz-Irastorza G, Khamashta MA, Nelson-Piercy C, Hughes GR. Lupus pregnancy: is heparin a risk factor for osteoporosis?. *Lupus* 2001;10:597–600.
165. Deruelle P, Coulon C. The use of low molecular weight heparins in pregnancy – how safe are they?. *Curr Opin Obstet Gynecol* 2007;19:573–77.
166. Clowse ME, Magder L, Witter R, Petri M. Hydroxychloroquine in lupus pregnancy. *Arthritis Rheum* 2006;54:3640–47.
167. Khamashta MA, Buchanan NM, Hughes GR. The use of hydroxychloroquine in lupus pregnancy: the British experience. *Lupus* 1996;5(Suppl. 1):S65–6.

168. Klinger G, Morad Y, Westall CA *et al.* Ocular toxicity and antenatal exposure to chloroquine or hydroxychloroquine for rheumatic diseases. *Lancet* 2001;358:813–14.
169. Motta M, Tincai A, Faden D *et al.* Follow-up of infants exposed to hydroxychloroquine given to mothers during pregnancy and lactation. *J Perinatol* 2005;25:86–89.
170. Pradat P, Robert-Gnansia E, Di Tanna GLand contributors to the MADRE database *et al.* First trimester exposure to corticosteroids and oral clefts. *Birth Defects Res A Clin Mol Teratol* 2003;67:968–70.
171. Park-Wyllie L, Mazzotta P, Pastuszak A *et al.* Birth defects after maternal exposure to corticosteroids: prospective cohort study and meta analysis of epidemiological studies. *Teratology* 2000;62:385–92.
172. Day RO. Sulphasalazine. In: WN Kelley, ED Harris, S Ruddy, CB Sledge, eds. Textbook of Rheumatology, fifth ed.Vol. I. Philadelphia, PA: WB Saunders; 1997:741.
173. Janssen NM, Genta MS. The effects of immunosuppressive and anti-inflammatory medication on fertility, pregnancy, and lactation. *Arch Intern Med* 2000;160:610–19.
174. Ostensen M. Treatment with immunosuppressive and disease modifying drugs during pregnancy and lactation. *Am J Reprod Immunol* 1992;28:148–52.
175. Ferrero S, Ragni N. Inflammatory bowel disease: management issues during pregnancy. *Arch Gynecol Obstet* 2004;270:79–85.
176. Freeman JG, Reece VA, Venables CW. Sulphasalazine and spermatogenesis. *Digestion* 1982;23:68–71.
177. Jarnerot G, Into-Malmberg MB. Sulphasalazine treatment during breast feeding. *Scand J Gastroenterol* 1979;14:869–71.
178. Branski D, Kerem E, Gross-Kieselstein E, Hurvitz H, Litt R, Abrahamov A. Bloody diarrhea-a possible complication of sulfasalazine transferred through human breast milk. *J Pediatr Gastroenterol Nutr* 1986;5:316–17.
179. de Boer NK, Jarbandhan SV, de Graaf P, Mulder CJ, van Elburg RM, van Bodergraven AA. Azathioprine use during pregnancy: unexpected intrauterine exposure to metabolites. *Am J Gastroenterol* 2006;101:1390–92.
180. Goldstein LH, Dolinsky G, Greenberg R *et al.* Pregnancy outcome of women exposed to azathioprine during pregnancy. *Birth Defects Res A Clin Mol Teratol* 2007;79:696–701.
181. Ramsey-Goldman R, Schilling E. Immunosuppressive drug use during pregnancy. *Rheum Dis Clin North Am* 1997;23:149–67.
182. French AE, Koren Gand the Motherisk Team. Effect of methotrexate on male fertility. *Can Fam Physician* 2003;49:577–78.
183. Temprano KK, Branlamudi R, Moore TL. Antirheumatic drugs in pregnancy and lactation. *Semin Arthritis Rheum* 2005;35:112–21.
184. Nandakumaran M, Eldeen AS. Transfer of cyclosporine in the perfused human placenta. *Dev Pharmacol Ther* 1990;15:101–5.
185. Venkataramanan R, Koneru B, Wang CC, Burckart GJ, Caritis SN, Starzl TE. Cyclosporine and its metabolites in mother and baby. *Transplantation* 1988;46:468–69.
186. Di Paolo S, Monno R, Stallone G *et al.* Placental imbalance of vasoactive factors does not affect pregnancy outcome in patients treated with Cyclosporine A after transplantation. *Am J Kidney Dis* 2002;39:776–83.
187. Ostensen M, Khamashta M, Lockshin M *et al.* Anti-inflammatory and immunosuppressive drugs and reproduction. *Arthritis Res Ther* 2006;8:209.
188. Cockburn I, Krupp P, Monka C. Present experience of Sandimmun in pregnancy. *Transplant Proc* 1989;21:3730–32.
189. Armenti VT, Ahlswede KM, Ahlswede BA *et al.* National Transplantation Pregnancy Registry – outcomes of 154 pregnancies in cyclosporine-treated female kidney transplant recipients. *Transplantation* 1994;57:502–6.
190. Bar Oz B, Hackman R, Einarson T, Koren G. Pregnancy outcome after cyclosporine therapy during pregnancy: a meta-analysis. *Transplantation* 2001;71:1051–55.
191. Sifontis NM, Coscia LA, Constaninescu S, Lavelanet AF, Moritz MJ, Armenti VT. Pregnancy outcomes in solid organ transplant recipients with exposure to mycophenolate mofetil or sirolimus. *Transplantation* 2006;82:1698–702.
192. Perez-Aytess A, Ledo A, Boso V *et al.* In utero exposure to mycophenolate mofetil: a characteristic phenotype?. *Am J Med Genet* 2008;146:1–7.
193. Downs SM. Induction of meiotic maturation in vivo in the mouse by IMP dehydrogenase inhibitors: effects on the developmental capacity of ova. *Mol Reprod Develop* 1994;38:293–302.
194. Vasiliauskas EA, Church JA, Silverman N, Barry M, Targan SR, Dubinsky MC. Case report: evidence for transplacental transfer of maternally administered infliximab to the newborn. *Clin Gastroenterol Hepatol* 2006;4:1255–58.
195. Skomsvoll JF, Wallenius M, Koksvik HS *et al.* Drug insight: anti-tumor necrosis factor therapy for inflammatory arthropathies during reproduction, pregnancy and lactation. *Nature Clin Pract Rheumatol* 2007;3:156–64.
196. Carter JD, Vareriano J, Vasey FB. Tumor necrosis factor-alpha inhibition and VATER association: a causal relationship. *J Rheumatol* 2006;33:1014–17.
197. Roux CH, Brocq O, Breuil V, Albert C, Euller-Ziegler L. Pregnancy in rheumatology patients exposed to anti-tumor necrosis factor (TNF)-alpha therapy. *Rheumatology (Oxford)* 2007;46:695–98.
198. Treacy G. Using an analogous monoclonal antibody to evaluate the reproductive and chronic toxicity potential for a humanized anti-TNFalpha monoclonal antibody. *Hum Exp Toxicol* 2000;19:226–28.
199. Katz JA, Antoni C, Keenan GF, Smith DE, Jacobs SJ, Lichtenstein GR. Outcome of pregnancy in women receiving infliximab for the treatment of Crohn's disease and rheumatoid arthritis. *Am J Gastroenterol* 2004;99:2385–92.
200. Kimby E, Sverrisdotti A, Elinder G. Safety of rituximab therapy during the first trimester of pregnancy: a case history. *Eur J Haematol* 2004;72:292–95.
201. M. Herold, S. Scnohr, H. Bittrich, Efficacy and safety of a combined rituximab chemotherapy during pregnancy, J. Am. Acad. Dermatol. 52 (Suppl. 2) AB8:155–208

Clark, *et al*, reply

To the Editor:

We read with interest Dr. Carp's comments regarding the HepASA trial. The questions he raises about our inclusion criteria and conclusions are those that have surrounded therapeutic trials for recurrent pregnancy loss (RPL) with or without antiphospholipid antibodies (aPL) for decades.

Dr. Carp suggests that stratification for this patient population should include age and number of previous losses. The issue of evaluating and treating women with a history of 2 versus 3 losses continues to be debated, and evidence supporting both contentions is available and used by investigators according to their convictions. As noted, we and others have observed that causes of RPL are similar in women with 2 or 3 losses[1,2]. Brigham, *et al*, in a longitudinal study of 325 patients with idiopathic RPL, found that women with a history of 2 versus 3 previous losses had the same chances of success in a subsequent pregnancy (76% vs 79%)[3]. Intuitively, therefore, if, in the absence of an identifiable etiology, there is no difference in subsequent pregnancy outcome and if there is no difference in the distribution of known etiologies of loss between women with 2 versus 3 losses, it is acceptable to include both groups without stratifying by number of losses. Indeed, our analysis supported this inference, as we found no difference in outcome when comparing women with 2 versus 3 previous losses[4]. As Dr. Carp noted, and as supported by Brigham's large prospective study[3], maternal age has a profound influence on future pregnancy success, and appears to be the most significant factor in predicting subsequent pregnancy outcome in women with RPL. Age was not a discriminating factor in our trial, as there was no significant difference between the 2 treatment groups (33.8 vs 34.6 yrs). The issues of number of losses and age have already been thoroughly addressed with regard to this population. Our trial design incorporated 2 of the strata that have not yet been adequately investigated with regard to the treatment efficacy of heparin and aspirin (ASA) for RPL: the effect of aPL positivity and a history of early versus late losses.

Regarding the inclusion of women positive for antinuclear antibodies (ANA) as well as aPL in our trial, we discussed the controversial status of the decision in our report. However, as we stratified the 2 treatment groups on the presence of aPL positivity, we were able to analyze the (apparently) more interesting aPL subgroups without interference from the inclusion of ANA-positive women. We reiterate that investigating a broader spectrum of autoantibodies than just aPL for women with RPL in the absence of connective tissue disease may be important, as it is emerging that in patients with primary antiphospholipid syndrome (APS), there may be an inflammatory component to the RPL with an etiology that remains to be elucidated[5].

Dr. Carp mentions 3 reports that have evaluated heparin and ASA versus ASA alone, and cites them as a "more closely defined group of women with antiphospholipid syndrome and 3 or more miscarriages." Unfortunately, this is not the case. Kutteh, *et al*[6] specifically excluded women with the lupus anticoagulant (a classification criterion for APS) and studies by both Rai, *et al*[7] and Farquharson, *et al*[8] included women with such low levels of anticardiolipin antibodies (IgG > 5 and IgM > 3[7]; and IgG > 9 and IgM > 5[8]) that they would not have fulfilled classification criteria for APS regardless of the number of miscarriages. As discussed in our report, Rai and Kutteh's studies, both completed and published more than 10 years ago, have the lowest birthrates in their ASA-only treatment groups in the literature, and they are the only studies that found a significant difference in pregnancy outcome with the addition of heparin. Because they were the earliest trials reported for this population, the results became the basis for clinical decision-making that has not assimilated new data published over the intervening decade. Subsequent trials that have reported much higher success rates in their ASA-only treatment arms and detected no significant improvement in pregnancy outcome with anticoagulant therapy have been largely disregarded or misconstrued. For example, in his letter, Dr. Carp cites Farquharson, *et al*'s report[8] as evidence supporting the continued use of heparin and ASA despite their conclusion to the contrary — that no benefit was conferred by the addition of heparin to ASA treatment.

Regardless of treatment regimen, number of prior losses, history of early versus late losses, or aPL positivity, almost 80% of our patients had a live birth. While we clearly conceded the weaknesses in our trial, we stand by our contention that for women with RPL, aPL, and no history of thrombosis, there is insufficient evidence for recommendations for thromboprophylaxis in pregnancy. Unfortunately, this treatment has become entrenched and continues as an example of eminence- rather than evidence-based medicine.

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REFERENCES

1. American College of Obstetricians and Gynecologists. Management of recurrent early pregnancy loss. ACOG Practice Bulletin No. 24. 2001;24:1-8.
2. Jaslow CR, Carney JL, Kutteh WH. Diagnostic features identified in 1020 women with 2 versus three or more recurrent pregnancy losses. Fertil Steril 2009 Mar 30. [Epub ahead of print]
3. Brigham SA, Conlon C, Farquharson RG. A longitudinal study of pregnancy outcome following idiopathic recurrent miscarriage. Human Reprod 1999;14:2868-71.
4. Laskin CA, Spitzer KA, Clark CA, et al. Low molecular weight heparin and aspirin for recurrent pregnancy loss: Results from the randomized, controlled HepASA Trial. J Rheumatol 2009;36:279-87.
5. Hughes G. Hughes syndrome: the antiphospholipid syndrome — a clinical overview. Clin Rev Allergy Immunol 2007;32:3-12.
6. Kutteh WH. Antiphospholipid antibody-associated recurrent pregnancy loss: treatment with heparin and low dose aspirin is superior to low dose aspirin alone. Am J Obstet Gynecol 1996;174:1584-9.
7. Rai R, Cohen H, Dave M, Regan L. Randomized controlled trial of aspirin and aspirin plus heparin in pregnant women with recurrent miscarriage associated with phospholipids antibodies. BMJ 1997;314:253-7.
8. Farquharson RC, Quenby S, Greaves M. Antiphospholipid syndrome in pregnancy: A randomized controlled trial of treatment. Obstet Gynecol 2002;100:408-13.

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CHAPTER

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Pregnancy and Reproductive Concerns in Systemic Lupus Erythematosus

Carl A. Laskin, Karen A. Spitzer, Christine A. Clark

s0010

INTRODUCTION

p0010 Systemic lupus erythematosus (SLE) is a multisystem, autoimmune disease predominantly affecting women in their child-bearing years, with a female-to-male ratio of 9:1. It is therefore not surprising that those physiological states associated with hyperestrogenicity or other changes in sex hormone levels may affect disease status. The hormonal changes associated with pregnancy may indeed have a profound influence on lupus disease activity [1, 2]. Management of pregnant women with SLE presents a challenge to all attending physicians whether rheumatologists, general internists, medical subspecialists, or obstetricians. A coordinated management plan is essential for attending to both the medical and obstetrical needs of the patient. Ideally a woman with lupus should be evaluated prior to a pregnancy to ensure that there is no medical contraindication. On too many occasions, a patient may first present in a medical office pregnant either with active disease or on unsafe medications. Critical decisions must be made at these times that may be difficult emotionally and ethically.

p0015 In addition to pregnancy, other issues that are relevant to the woman with SLE include contraception and the investigation and management of infertility. This chapter will concentrate on pregnancy issues but will also address these broader issues concerning reproduction.

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PHYSIOLOGICAL CHANGES RELEVANT TO WOMEN WITH UNDERLYING SLE

p0020 Pregnancy is accompanied by major physiological changes, which may adversely affect the woman with underlying SLE. In addition, such changes will affect numerous clinical and lab parameters complicating the assessment of disease activity in the pregnant woman with SLE. Among those systemic changes relevant to the woman with SLE are: cardiovascular with potential cardiac, renal, and hypertensive complications; hematologic including changes in blood volume and control mechanisms of coagulation such as changes in platelet counts, clotting factor levels, and regulatory proteins; immunologic including the regulation of both humoral and cellular immunity, complement kinetics, and the development of immune complexes; and hormonal with changing levels of estrogen, progesterone, prolactin, and protein-hormone binding globulins [3].

Cardiovascular

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p0025 Associated with pregnancy are dramatic changes in maternal hemodynamics due to increases in blood volume, heart rate, stroke volume, and a decrease in systemic vascular resistance [3, 4]. Cardiac output increases by 30–50% with a 20% increase in heart rate and a 5- to 10-mmHg decrease in mean arterial pressure in pregnancy. The lupus patient with underlying cardiac, renal, or other vascular complications of the disease may be at risk of further major organ deterioration during pregnancy.

Hematologic

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p0030 The hematologic changes that occur normally in pregnancy compound the changes in the cardiovascular system. Plasma volume increases 30–50% beyond that seen in the nonpregnant state with the red cell mass increasing 20–40% [5, 6, 7]. Again, such changes may not bode well for the lupus patient with cardiovascular or renal compromise. Other alterations include an

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increase in white blood cell count and a mild decrease in platelet count [8, 9]. Although the WBC increases during pregnancy, the absolute lymphocyte count does not change. In contrast, changes in platelet counts may manifest as asymptomatic thrombocytopenia in the third trimester in many normal pregnancies. These changes observed in the CBC may confound the assessment of disease activity in the pregnant woman with SLE.

p0035 It is well appreciated that pregnancy is a prothrombotic state due to increases in the levels of several clotting factors (factors II, VII, VIII, X, and von Willebrand); a decrease in the levels or activity of naturally occurring anticoagulants such as Protein S as well as an increase in activated Protein C resistance; and an increase in the activity of fibrinolytic inhibitors [10–14]. Owing to these changes in pregnancy, there may be an increased theoretical risk of thrombotic events in those with antiphospholipid antibodies. Supporting data regarding such an increased risk is still lacking.

s0030 Immunologic

p0040 During pregnancy, alterations in immune function occur affecting lymphocyte function, humoral immunity, and the inflammatory response. Awareness of these changes is of paramount importance in the assessment of a patient with known SLE. Human pregnancy is associated with an increase in immune suppressor activity, leading to a decrease in humoral B cell function. In lupus there is dysregulation of the immune response, leading to an impairment of suppressor activity, resulting in polyclonal B cell activation.

p0045 Pregnancy-specific proteins such as α-fetoprotein, β_1-glycoprotein, α_2-macroglobulin, and others suppress lymphocyte function, as do increases in endogenous corticosteroids. Although their roles are not completely understood, interleukins-1 and -3 (IL-1, IL-3), tumor necrosis factor α (TNF-α), interferon-γ (IFN-γ), granulocyte–macrophage colony-stimulating factor (GM-CSF), and other cytokines may be critical in sustaining pregnancy [15]. This is relevant in that the production or metabolism of some cytokines is thought to be abnormal in rheumatic illness.

p0050 Therefore, the superimposition of pregnancy on the immunoregulatory abnormalities present in a woman with lupus will alter the immune environment. The interaction of these two altered immune states must be appreciated in order to account for changes in disease activity.

s0035 Hormonal

p0055 Estrogen, progesterone, prolactin, and other hormone levels rise in pregnancy [16]. Estrogens upregulate and androgens downregulate T-cell responses and immunoglobulin synthesis [17]. IL-1, IL-2, IL-6, and TNF-α can be regulated by sex hormones. Since circulating and local levels of cytokines may be abnormal in SLE, it is likely that these hormonally induced alterations in immune function may affect disease activity in the pregnant woman with SLE.

THE IMMUNOLOGY OF IMPLANTATION, PREGNANCY, AND LABOR AND DELIVERY s0040

The fundamental theory of the establishment of pregnancy has been the Th-1/Th-2 paradigm [1]. Helper T-cell clones can be divided into two phenotypes that secrete a distinct cytokine pattern [18]. During pregnancy, there is an overall suppression of Th-1-mediated cellular immunity and an enhancement of Th-2-mediated humoral immunity. The maintenance of pregnancy requires the downregulation of the pro-inflammatory Th-1 cytokines (TNF-α, IFN-γ, and IL-2) and the upregulation of anti-inflammatory Th-2 cytokines (IL-4, IL-6, and IL-10). During embryo implantation, uterine epithelial cells surrounding the blastocyst undergo apoptosis. These apoptotic cells are then taken up by macrophages found in excess at the implantation site. The uptake of the apoptotic cells into the macrophages promotes the secretion of Th-2 cytokines and suppresses the release of Th-1 cytokines including TNF-α from the macrophages. The Th-2-rich anti-inflammatory environment surrounds and protects the developing embryo. Therefore, pregnancy appears to be a Th-2 phenomenon. Recent data, however, suggest that this paradigm may be overly simplistic. Although TNF-α and IFN-γ have been implicated in failed implantation or early pregnancy loss, it now appears that small quantities of these cytokines may be necessary for the successful implantation of the embryo. Moreover, it may be that Th-1 activity both accompanies and predominates over Th-2-mediated events during the early implantation period, and premature and term labor. Th-1 activity plays an important role in the promotion of the Th-2 response, regulation of the placentation process, defense against infections, and initiation of delivery. The new paradigm should more properly be thought of as "Th-1–Th-2 cooperation" rather than a Th-2-dominant phenomenon. p0060

THE PLACENTA s0045

The placenta is often the target organ culminating in adverse pregnancy outcome in women with SLE. Abnormalities in placentation, uteroplacental vascular insufficiency, and placental infarction may be the underlying cause of intrauterine growth restriction (IUGR), p0065

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preeclampsia, and preterm delivery often described as complicating factors in lupus pregnancies [19, 20]. Therefore, an understanding of normal placental physiology and morphology is necessary to appreciate the pathological changes that may occur in the placenta during pregnancy.

p0070 The placenta brings maternal and fetal blood in close relationship. The placenta is partly of fetal origin (the trophoblast) and partly of maternal origin (transformation of the uterine mucosa). The fetal trophoblast ensures implantation. It is composed of two layers: the inner cellular layer (cytotrophoblast) and the exterior syncytial layer (syncytiotrophoblast). The placenta is well defined by the 3rd month. At term it weighs 500 g and is 20 cm in diameter. The human placenta is villous, hemochorial, and chorioallantoic, meaning that the placental villi are bathed directly in maternal blood and are traversed by vessels coming from the allantoic circulation of the fetus. While maintenance of pregnancy prior to implantation is assured by both ovarian and pituitary hormones, after implantation the placenta assumes an important role in hormone production: chorionic gonadotrophins are discernible several days after nidation, peak at the 60th day, and then fall to low levels until completion of pregnancy [21].

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MANAGEMENT OF PREGNANT WOMEN WITH SLE

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General Principles

p0075 In any woman with an underlying medical problem, pregnancy should be planned in advance to minimize the risks to mother and fetus, thereby maximizing the probabilities of a successful outcome. A prepregnancy medical and obstetrical evaluation represents the ideal management plan. In the case of rheumatic diseases, specifically SLE, a rheumatologist should assess the patient when a pregnancy is being considered. The objective of the assessment is to note the manifestations of the underlying disease, the past history of exacerbations and past and current medications. With this approach, the attending physician will then be able to document: (1) the clinical profile; (2) the laboratory profile; (3) the frequency and pattern of the most recent disease flare; and (4) the current medications.

- Clinical profile. This profile is constructed from the history and physical examination. In addition, the past history is utilized to record how the disease initially presented and the usual manifestations of a flare.
- Laboratory profile. The laboratory tests that best reflect the patient's disease status are used as indicators of disease activity during a pregnancy. The profile is constructed from the current laboratory tests and any relevant serology; as well as noting which laboratory variables are associated with disease activity.
- Most recent disease flare. It is important to determine and document the most recent exacerbation of the disease as well as the severity. The timing of a pregnancy will determine the safety of such an undertaking. Appropriate counseling of the patient can then be undertaken. This information should be incorporated into the patient's clinical profile.
- Medications. A detailed history of current medications is essential to provide appropriate counseling to the patient regarding the timing of a pregnancy. Many antirheumatic drugs are unsafe in pregnancy, and because many are also long-acting, they may have to be discontinued months prior to conception.

The Prepregnancy Evaluation

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p0100 Women with underlying SLE should consider a planned pregnancy to ensure their safety as well as the best outcome for themselves and their newborn. Whenever possible, women with SLE contemplating pregnancy should be evaluated prior to conception to determine disease status and activity. The clinician should determine the patient's clinical and laboratory profile, date of most recent flare of the disease, and current medications. Using a methodical approach, the physician may grasp the subtle nuances of the underlying disease, permitting better distinction of disease activity and the physiological or pathophysiological changes associated with pregnancy.

p0105 Pregnancy in any woman with an underlying medical problem should be managed by both an internist/subspecialist and obstetrician/perinatologist. In the case of SLE, that internist should be a rheumatologist or at the very least an internist familiar with the management of lupus. The prepregnancy evaluation should form the basis of a recommendation to the patient regarding the medical management plan. Appropriate counseling regarding the potential effect of the disease on the pregnancy and neonate, and similarly the effect of the pregnancy on the disease should be provided.

p0110 The prepregnancy evaluation must include a detailed history, noting presenting and usual manifestations of the disease, which will assist in distinguishing a disease flare from other pregnancy-related complications. The timing of the most recent flare as well as documenting a frequency of flare pattern will indicate the safety of undertaking a pregnancy at a particular time. The current medications as well as a recent medication history are essential as many drugs used to treat SLE

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are relatively or absolutely contraindicated in pregnancy and some require a specific washout period of time prior to conception. The physical examination must be documented, especially any abnormalities to ensure an accurate baseline clinical evaluation prior to pregnancy. Laboratory investigations should include baseline CBC, liver function tests, renal function, and serology. A baseline urine evaluation is essential and should include not only a dipstick with microscopic evaluation of the sediment but a protein/creatinine ratio on a spot sample or 24-h collection. A recommendation and plan should be discussed with the patient and relayed to all physicians involved in the management. Even if the opinion is not to undertake pregnancy at this time, the physician should present to the patient the reasons for the negative recommendation as well as the goals that need to be achieved before a favorable recommendation can be given. Booking a reassessment visit may prevent the occurrence of an unplanned pregnancy by a woman who simply decides to proceed despite the risks.

p0115 Once the decision has been made to proceed with a pregnancy, the management plan should be discussed with the patient. This will involve presenting the risks of potential problems, proper use of medications and their safety, frequency of visits and monitoring specifics, as well as a recommendation for obstetrical management. For individuals with complicated but stable disease, who will undertake a pregnancy in the near future, a prepregnancy obstetrical assessment should be considered. The ideal obstetrician is a perinatologist familiar with lupus in pregnancy. The perinatologist should be informed of the medical management plan and the obstetrical and medical prenatal assessments should be coordinated to minimize the inconvenience to the patient and maximize the lines of communication among all involved.

s0065 Prenatal Assessments

p0120 The first prenatal visit will include a complete history, physical exam, and laboratory assessment, which will be compared to that obtained at the prepregnancy evaluation. This will serve as the baseline for future assessments during the pregnancy, noting any clinical or lab evidence consistent with disease activity. The frequency of visits should be at minimum once per trimester in those cases of full remission with minor organ disease. In those women with a history of major organ disease or any evidence of active lupus, the frequency should be at least once monthly or coincide with obstetrical visits. Where the patient shows any medical instability, visits will be more frequent or the patient may require hospital admission. Regular, frequent medical assessments are the norm to minimize the probability of an unexpected disease exacerbation.

The Effect of Pregnancy on SLE s0070

p0125 In all pregnant women with an underlying medical problem, the physician must view the situation as the effect of the pregnancy on the disease and the effect of the disease on the pregnancy. The former scenario is more medical, whereas the latter scenario is related to things obstetrical. This is the exact situation in SLE and all physicians involved in patient management must be cognizant of this "two-way street."

p0130 There is some consistency in the response of SLE to pregnancy, but some patients do not "behave" as expected. As early as 1952, it was noted that some women with SLE flare during pregnancy [22]. Other studies have also noted an increase in flares during pregnancy [23–26]. The more recent literature, however, notes that the frequency of disease exacerbation during pregnancy and postpartum is less than that reported earlier [27, 28]. In a case–control prospective study comparing pregnant and nonpregnant women with similar manifestations of SLE, Lockshin *et al.* found no increase in flares during pregnancy [29, 30]. In contrast, Petri concluded that pregnancy was associated with an increased rate of disease flares in her population with a frequency of 1.63 per person years compared to 0.64–0.65 in a postpartum group or in nonpregnant controls [31]. Ruiz-Irastorza *et al.* observed findings similar to Petri with a 65% flare rate during pregnancy compared to 42% in the control group [32]. Recent studies by Cortes-Hernandez *et al.* found that 33% of their lupus patients flared during pregnancy, with 26% in the second trimester and 51% postpartum [33]. The major predictors of a flare were the discontinuation of antimalarial treatment, a history of more than three flares before the pregnancy, and a SLEDAI (Systemic Lupus Erythematosus Disease Activity Index) score of 5 or more during these flares. In one study of 46 women with SLE who underwent 61 pregnancies, Urowitz *et al.* observed no increased frequency of lupus flares, using the SLEDAI, during pregnancy compared with controls [34]. Indeed there was a reduced chance of flare during a pregnancy if the patient had inactive disease for 6 months prior to conception. Clearly there is a lack of consensus among these studies. This may be due to dissimilar entry criteria, differing definitions of a flare, distinct patient populations, and differing control groups [31, 32]. Despite the lack of consensus regarding the relationship between SLE flare and pregnancy, it is clear that many patients do well during pregnancy and that vigilance for flare on the part of the treating physician is always necessary. Modifications have been suggested for measuring SLE flare during pregnancy using the SELENA SLEDAI (SLEPDAI, systemic lupus erythematosus pregnancy disease activity index), the LAI (LAI-P, lupus activity index pregnancy), or the

SLAM-R (m-SLAM, modified systemic lupus activity measure) and have been tested [35, 36].

p0135 The incidence of adverse pregnancy outcomes in women with SLE is increased. In a retrospective analysis, 555 women with SLE had an adverse outcome apart from manifestations of SLE compared to a group of 600,000 controls [37]. These outcomes included hypertension, renal disease, preterm delivery, nonelective cesarean section, postpartum hemorrhage, and delivery-related deep venous thrombosis. Clark *et al.* noted 38.9% of women with SLE had a preterm delivery (before 37 weeks gestation) in a group of 72 pregnancies [38]. The observation of preterm delivery was associated with disease activity and the presence of IgG anticardiolipin antibody.

s0075 ***Renal Disease***

p0140 Renal function may decline during pregnancy in those with renal disease, but it is determined, not surprisingly, by the severity of the underlying renal dysfunction. A permanent decline will occur in up to 10% of women with glomerular filtration rates initially normal or mildly reduced (serum creatinine less than 132 μmol/l). Patients with hypertension are much more likely to have progressive disease regardless of pregnancy status [39–42]. In those with moderate renal insufficiency (serum creatinine, 132–255 μmol/l), the serum creatinine declines over the first half of pregnancy similar to that in those without renal disease, but then may increase significantly higher and above that observed at the onset of pregnancy [43, 44]. This decline in renal function may be irreversible, especially in those with hypertension [45].

p0145 Any renal disease, including lupus glomerulonephritis, predisposes patients to preeclampsia. There is some disagreement whether patients with renal SLE do less well than those with other forms of glomerulonephritis. In general, those with preserved renal function and minimal hypertension do well. Even those with severe renal disease often have surprisingly good pregnancy outcomes. Normal urinary protein in pregnancy may be as high as 500 mg/day. Modest increases in urinary protein (particularly in patients with fixed proteinuria), accompanied by proportional increases in glomerular filtration in mid- to late pregnancy and not accompanied by other signs of active lupus, likely do not indicate lupus nephritis and need not be treated. Our experience leads us to expect an increase in proteinuria after 20 weeks in almost all patients who have had any renal involvement. The increase in proteinuria is related to the normal increase in plasma volume seen in pregnancy, which appears to overwhelm the filtering capacity of the previously damaged glomeruli. In patients with marked increases in urinary protein, with or without hypertension, it may be impossible to distinguish between preeclampsia and active lupus nephritis, and may therefore be necessary to treat if delivery is not likely in the immediate future. Isolated hypocomplementemia may suggest increased vigilance but by itself does not warrant intervention.

p0150 Renal disease may flare during a pregnancy, which may respond to treating with corticosteroids or increasing the dosage of administered steroids [32]. Studies have been inconsistent in finding deterioration in renal function associated with pregnancy [27, 37, 46–50]. Tozman *et al.* noted no recurrence of renal disease in 11 of 18 patients with similar findings by Jungers *et al.* and Huong *et al.* All noted that the best prognosis was associated with remission of the disease at pregnancy onset. Furthermore, they all noted a higher risk of preeclampsia and premature birth than expected. In contrast, others noted an increase of renal flare in pregnancy [48, 49]. These authors and others also conclude that the only predictor of a favorable maternal outcome in a pregnancy is quiescence of renal disease [34].

Renal Disease and Preeclampsia s0080

p0155 Preeclampsia is a syndrome occurring after 20 weeks gestation. The first manifestations are hypertension and proteinuria, but other clinical features may include headache, visual disturbances, epigastric pain, thrombocytopenia, and abnormalities in liver function tests [51]. The underlying pathogenesis is a microangiopathy affecting the brain, liver, kidney, and placenta [52]. The condition often precipitates preterm delivery owing to fetal compromise characterized by IUGR due to placental insufficiency. The inciting event actually occurs early in pregnancy with abnormalities in the development of the placental vasculature leading to underperfusion. The hypoperfusion then leads to the production of antiangiogenic factors, which upon release into the maternal circulation, adversely affect endothelial cell function, resulting in the manifestations noted above.

p0160 Preeclampsia is not uncommon in lupus pregnancies occurring in approximately 13% of such patients [53]. In those with underlying renal disease, the risk of developing preeclampsia may be much higher with a reported frequency that may be as high as 66% [54]. Superimposed on the increased risk associated with SLE and renal disease is an even greater risk in those patients with antiphospholipid antibodies (aPL), diabetes mellitus, or preeclampsia having occurred in a prior pregnancy [55].

p0165 A major diagnostic challenge to the clinician is to distinguish preeclampsia from a lupus flare during a pregnancy. Defining the clinical and laboratory profile of the patient before pregnancy will often assist in distinguishing these two conditions. If there are clinical features of active lupus and positive serology, this is

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likely a lupus flare. Depressed complement levels are characteristic of active lupus, whereas elevated complement levels are seen in pregnancy and usually do not change in preeclampsia. Although proteinuria may be seen both in preeclampsia and lupus nephritis, the presence of active sediment is a feature of active nephritis and not preeclampsia. Thus, a simple microscopic evaluation of the urine may be extremely informative, although it is acknowledged that Class V membranous glomerulonephritis can be associated with a totally benign sediment. Elevated liver function tests and uric acid with thrombocytopenia and decreased urinary excretion of calcium are characteristic of preeclampsia, whereas thrombocytopenia alone or, if there is renal insufficiency, elevated serum urate can be seen in active SLE. Knowledge of your patient and how her disease has manifested itself in the past will assist immeasurably in correctly assessing this difficult diagnostic situation.

s0085 ***Hematologic Disease***

s0090 THROMBOCYTOPENIA

p0170 Thrombocytopenia is common during a pregnancy, especially in women with underlying SLE. There are a number of causes of thrombocytopenia, which may be unrelated to the underlying autoimmune disease. As is the case in any pregnant woman with a low platelet count, a methodical approach to the differential diagnosis must be undertaken lest the clinician erroneously attribute the thrombocytopenia to active lupus. Management should be directed to the underlying cause without simply assuming lupus to be the culprit. Platelet counts in normal pregnancies may be toward the lower limit of normal but in general, they remain within the normal range throughout pregnancy [56–59].

p0175 Before concluding that thrombocytopenia is indeed present, be certain that the finding is not merely a spurious lab result due to EDTA-induced platelet aggregation. Once ruled out, the differential diagnosis should be undertaken in the pregnant woman with underlying SLE with consideration of the following possibilities:

1. Gestational thrombocytopenia is typically observed late in gestation with counts 70–100 $\times$ 10^9/l. The condition is benign without fetal/neonatal thrombocytopenia, and resolves after delivery [60–62]. Should this occur in a woman with SLE, close follow-up is necessary to ensure the platelet count stabilizes at a safe level and is not a manifestation of active lupus.
2. Thrombocytopenia occurring after 25 weeks is most often due to preeclampsia. Platelet counts are lower in preeclampsia than that seen in gestational thrombocytopenia, with an incidence of 15% in women who develop preeclampsia. Preeclampsia tends to worsen as pregnancy progresses, is associated with worsening fetal health, and remits after delivery, although not always immediately. It is not associated with other signs or symptoms of active SLE. Biochemical and clinical manifestations of preeclampsia or the HELLP syndrome (hemolysis, elevated liver enzymes, low platelet count) may be present. Treatment directed at early delivery is usually indicated. Since preeclampsia occurs with greater frequency in women with SLE, the clinician can expect thrombocytopenia more often in the pregnant woman with SLE. Distinction between preeclampsia and a lupus flare may be problematic but it is an important distinction to make nonetheless. The clinical and lab assessment as discussed above is necessary to help clarify the diagnosis.
3. Thrombocytopenia associated with an antiphospholipid antibody can occur at any time but is often seen early, usually before 15 weeks. The platelet count will reach a nadir greater than 50 $\times$ 10^9/l and remain constant throughout most of pregnancy. The condition typically remits after delivery, whether or not treatment is administered, and tends to recur with subsequent pregnancies. It is unaccompanied by signs of SLE activity in other organ systems; fragmented erythrocytes and increased lactic dehydrogenase (LDH) do not occur. Aspirin may reverse the thrombocytopenia [63].
4. Severe thrombocytopenia (often $<10 \times 10^9$/l) similar to that seen in idiopathic thrombocytopenic purpura may occur in lupus pregnancies but is more likely in those women with a history of thrombocytopenia as a major manifestation of SLE. When it occurs, it is often without other manifestations of active SLE and may occur at any time during the pregnancy. It will usually respond to high doses of prednisone and may require continuation of these higher doses. Intravenous gamma globulin can also be used as a temporizing measure, particularly to avoid splenectomy. The condition usually resolves postpartum but tends to recur in subsequent pregnancies. It is noteworthy that there is a 5–10% risk of neonatal thrombocytopenia (an example of passively acquired autoimmunity) in those born to mothers having had episodes of platelet counts below 50 $\times$ 10^9/L during the pregnancy with a greater risk if the mother has had a splenectomy [64–66]. Aspirin is contraindicated in those women with this type of thrombocytopenia.
5. In those women with other manifestations of SLE, thrombocytopenia may occur, usually in the moderate range of 50–120 $\times$ 10^9/l. These counts are characterized by persistence over time and

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responsiveness to prednisone. The low platelet count will be observed in those women where this is a known manifestation of lupus outside of the pregnant state.

p0205 No specific test clearly differentiates one type of thrombocytopenia from another in pregnant patients with SLE. Unfortunately, tests for platelet-associated immunoglobulin or for antiplatelet antibodies seldom help differentiate among the types of thrombocytopenia.

s0095 ANEMIA

p0210 Anemia is a common complication of SLE. The anemia "of chronic disease" is the most frequent attributed cause. A true autoimmune hemolytic anemia is uncommon, but does occur, with the risk being higher in those in whom there is a history of this being a manifestation of lupus [67]. SLE-induced anemia amplifies the normal dilutional anemia of pregnancy and, if severe, may indicate intervention using corticosteroids or blood transfusion.

s0100 ***Cutaneous Disease***

p0215 Normal pregnancy erythema, particularly of the face and hands, resembles that seen in active SLE. Skin areas where there has been prior lupus eruption frequently become more erythematous as cutaneous blood flow increases in pregnancy. With experience, distinguishing between active SLE cutaneous disease and pregnancy-induced change is not difficult.

s0105 ***Arthritis***

p0220 Joints previously affected by lupus arthritis often become painful and may even develop noninflammatory effusions during pregnancy, particularly late in gestation as ligamentous laxity occurs. The decision that a given complaint is due to active SLE rather than to physiologic changes of pregnancy depends on the demonstration that the arthritis is inflammatory. If extra-articular manifestations of active SLE are present, it is likely that the articular effusion represents active disease. If warranted, arthrocentesis may be informative with regard to leukocyte count and culture since there is no contraindication to this procedure during pregnancy.

s0110 ***Neurologic SLE***

p0225 Neurologic events during the course of pregnancy are rare. There are isolated reports but no cogent evidence that SLE neurologic events, including chorea and transverse myelitis, are induced or exacerbated by pregnancy [68–70]. There are also occasional reports of SLE patients suffering stroke postpartum, usually in the setting of coexisting antiphospholipid antibodies.

p0230 One of the more difficult diagnostic decisions concerns the occurrence of seizures late in pregnancy, when hypertension and renal failure are also present. The circumstances in which the events occur, the presence of other clinical and serologic evidence of active SLE, and response to therapy may all be required to make the distinction between active neurologic lupus and eclampsia. Treatment for both may be indicated.

The Effect of SLE on Pregnancy s0115

p0235 Adverse pregnancy outcome is more common in SLE than in any other rheumatic disease. Appropriate pre-pregnancy evaluation and counseling will maximize the probability of a successful outcome for both the mother and the neonate. Maintaining the viability of the pregnancy requires close collaboration between the obstetrician/perinatologist and the internist/rheumatologist.

p0240 The incidence of fetal wastage in SLE pregnancies has been reported in the past to be as high as 50%, including spontaneous abortion (miscarriage), prematurity, and stillbirth [23, 26, 31–34, 71–73]. A more recent analysis of long-term data over the past 40 years noted a decline in the spontaneous abortion rate from 50% to less than 20% [74]. Those risk factors associated with adverse outcomes included antiphospholipid antibodies, hypocomplementemia, and hypertension during pregnancy [33]. In addition, persistent, significant proteinuria (>0.5 g/day) would also likely contribute to adverse pregnancy and maternal outcomes. Although some studies report that infants born to mothers with SLE are small for gestational age, this has not been a consistent observation, even in cases where placental size is reduced.

p0245 The increased frequency of fetal loss in SLE may be due to several factors: (1) active lupus resulting in decidual vasculitis, which in turn compromises placental blood flow depriving the fetus; (2) trophoblast-reactive lymphocytotoxic antibodies; (3) anti-Ro/SSA or anti-La/SSB antibodies with their associated compromise of the fetal cardiac conduction system; and (4) antiphospholipid antibodies with resulting placental vascular thrombosis and insufficiency culminating in ischemic pregnancy loss.

Antiphospholipid Antibodies and Pregnancy s0120

p0250 Antiphospholipid antibodies (aPL) in low to moderate titer may be seen in 40–60% of lupus patients with active disease. The aPL family of antibodies includes IgG or IgM anticardiolipin antibodies (aCL) and a nonspecific *in vitro* inhibitor of coagulation often referred to as the lupus anticoagulant (LAC). Although the mere presence of aPL in a woman with lupus may not be associated with any particular manifestation, there may be an increased risk of adverse pregnancy

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outcome in such individuals. A review of ten studies comprising 554 women with SLE observed fetal loss more frequently in the presence of aPL (39–59%) compared to those without aPL (16–20%) [75]. In addition, aPL have been associated with preeclampsia and placental abruption [76–79]. Women with SLE and aPL and the associated clinical manifestations including thromboembolism and pregnancy wastage, are classified as secondary antiphospholipid syndrome (APS). Those with only aPL and an associated clinical feature are classified as primary APS since they lack any other feature of SLE.

p0255 LAC and aCL have often been used interchangeably to indicate the presence of antiphospholipid antibodies. While there is certainly a correlation between these two antibodies, they are not identical. Lockshin *et al.* reported that while both the LAC and aCL were associated with fetal loss, higher levels of aCL appear to be more predictive of fetal distress or fetal death among pregnant women with SLE [79]. However, other recent observations by Clark *et al.* and Salmon *et al.* have shown that the LAC may have a higher association with pregnancy loss and adverse outcome than aCL [80, 81]. The risk of fetal loss in women with circulating aPL has lately become controversial. Although some studies suggest that the presence of aCL or features of the antiphospholipid syndrome (APS) are associated with increased risk of pregnancy loss in women with SLE, others have not found this to be the case [73, 82–89]. Questions have arisen regarding the association of aPL with early vs. late pregnancy loss; the significance of IgM and IgA isotypes of aCL [90], and whether the presence of LAC or aCL in women with no history of thromboembolism or adverse pregnancy outcome is sufficient indication for intervention.

p0260 Treatment of pregnant women with APS, whether primary or secondary, has also become somewhat controversial. Although initial studies supported the use of prednisone and aspirin to promote live birth in a woman with a history of pregnancy loss and aPL, a double-blind, randomized controlled trial failed to show a benefit beyond placebo [91, 92]. Heparin (both unfractionated and more recently, low molecular weight) and aspirin have become the accepted treatment for the prevention of pregnancy loss in women with APS [93–98], although studies supporting the use of such therapy prior to 2000 contrast with those conducted after that date where ASA alone was found to be at least as efficacious as heparin with ASA [99–100].

s0125 Fetal and Neonatal Considerations

p0265 In general, term births from women with SLE are at no greater risk of congenital anomalies than those born to mothers without SLE. The major exception is those babies born to mothers possessing anti-Ro (SSA) and/or anti-La (SSB) antibodies. Although abnormalities occur in only 1–2% of the neonates [101, 102], the manifestations form collectively the neonatal lupus syndrome. Transient abnormalities include skin lesions, liver disease, and cytopenias. Each generally cleared by 6–8 months of life, coincident with the clearance of the maternal antibodies. However, cardiac anomalies including conduction defects and more rarely myocardial involvement are permanent, with the latter often fatal. The antibody may be found in up to 25–40% of women with SLE but may also be an isolated finding in the general population. Once a woman with anti-Ro/La antibodies has given birth to an infant with congenital heart block, the risk in a subsequent pregnancy is about 17% [103]

p0270 Maternal IgG-mediated thrombocytopenia may be transmitted to the fetus, but most infants born of thrombocytopenic mothers with SLE have normal platelet counts. Occasionally the IgG Coombs hemolytic antibody may be transmitted to and cause hemolysis in the fetus and newborn. The anti-dsDNA antibody, also transmitted to the infant, has no apparent pathologic effect. Recent studies on a subset of anti-dsDNA antibodies, anti-NR2, which cross-react with neuronal tissue, destroy such tissue by excitotoxicity and apoptosis [104, 105]. These maternal antibodies may cross the placenta and result in cognitive dysfunction in the offspring of mothers with the autoantibody. Although the antiphospholipid antibody is associated with placental insufficiency, intrauterine growth restriction, and fetal death, it does not usually cause abnormalities in the infant.

Summary of the Management of SLE During Pregnancy s0130

p0275 Ideally, lupus should be inactive or under excellent control on minimal therapy for at least 6 months prior to pregnancy. Should the disease flare during pregnancy, treatment must be instituted immediately using the safest, most effective regimen. Prednisone (being nonfluorinated and thereby limiting its ability to cross the placenta owing to inactivation by placental 11-beta dehydrogenase) has few adverse effects on the fetus and should be used if necessary. Other drugs with very good safety profiles in SLE include some nonsteroidal anti-inflammatory drugs (naproxen, ibuprofen), antimalarials, and azathioprine. Indications for the treatment of the pregnant patient with lupus do not differ from that when considering treatment in the nonpregnant woman. Clinical disease flares should be treated with the same dose of corticosteroids regardless of pregnancy status.

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s0135 ***Monitoring***

p0280 Both the obstetrician and internist should concurrently follow pregnant women with SLE. Efforts should be coordinated to avoid duplication of laboratory tests and inappropriate, overly liberal consultation with other medical specialties. Maternal clinical evaluation in addition to appropriate laboratory testing will determine disease activity. If the patient's serology is concordant with disease activity, then rising anti-dsDNA antibody and falling complement levels will be the markers of a disease flare. Be aware that complement levels in pregnancy are typically elevated, so a falling level should be noted, not just a low level. Validated measures of disease activity usually restricted to research protocols can be adapted for use in the clinic setting [106, 107]. Echocardiograms for fetuses of anti-Ro antibody-positive mothers should be initiated between 16 and 18 weeks with frequencies determined by the managing physicians but often are recommended once weekly [101].

s0140 ***Labor and Delivery***

p0285 The obstetrician should make the decision regarding the mode of delivery. Most patients with SLE will deliver vaginally. Corticosteroid supplementation should be administered at labor or prior to a cesarean section in women currently or recently using such medications. Of note, there is no rationale for prophylactically increasing the dose of corticosteroids to prevent a postpartum flare of the disease [106].

s0145 ***Postpartum***

p0290 Some women may flare postpartum, but it is usually those who had active disease at conception with continued activity throughout the pregnancy [108]. There is no indication for the routine use of adding or increasing corticosteroids just prior to delivery as a means of preventing an exacerbation of disease. The risk of a postpartum flare of lupus is approximately 20%, but the vast majority of patients remain as they were during the pregnancy. However, it is recommended that all patients be assessed shortly after delivery (4–6 weeks postpartum) and reassessed periodically over the following 3 months. Treatment of those women with active lupus postpartum is the same as in the case of nonpregnant women with SLE. However, if treatment is required and the woman is breast-feeding, it may be necessary for her to discontinue depending upon medications necessary to manage the disease.

s0150 ***Breast-Feeding***

p0295 The major concern in nursing the neonate is exposure to certain medications that may enter the breast milk. The underlying disease *per se* is not an issue in terms of the safety of the infant. If the disease is active, caution must be exercised due to some medications with which the mother may be treated. Prednisone (up to 30–40 mg/day), hydroxychloroquine, and short-acting nonsteroidal agents (naproxen, ibuprofen) are considered compatible with breast-feeding.

PHARMACOLOGIC TREATMENT OF SLE IN PREGNANCY s0155

The assessment of any pregnant patient with rheumatic disease or those contemplating pregnancy must include consideration of current medications and, in some cases, previous exposure to specific pharmacologic agents. Although most drugs may be safe while the couple is attempting to conceive, some must be discontinued during pregnancy and yet others require discontinuation some time prior to conception. Under ideal conditions, medications should be discontinued prior to pregnancy but not at the expense of the patient's well-being. An understanding of the woman's medical condition including disease status and any compromised organ function such as renal insufficiency must be taken into consideration when deciding upon the use of specific therapeutic agents. Moreover, the physician must have extensive knowledge of specific drugs or have knowledge of the resources available to obtain such information when recommending implementation or discontinuation of specific drugs. p0300

This section will address those agents commonly used in the treatment of SLE and its various manifestations. As evidence accumulates, recommendations regarding the safe use of particular drugs in pregnancy will change. Therefore, it is incumbent upon the clinician to be current with respect to the safe use of any of these agents in pregnancy. p0305

Nonsteroidal Anti-inflammatory Drugs (NSAIDs) s0160

NSAIDs are commonly used in the treatment of the arthritis manifested in many rheumatic diseases. Many are now available over the counter, making it of paramount importance that the obstetrician and internist be aware of all medications consumed by the patient whether prescribed or self-administered. p0310

Most NSAIDs are safe to use for couples attempting to conceive. However, for women, some agents can inhibit follicular rupture, preventing the release of the oocyte and thereby contributing to subfertility [109, 110]. This is not a common phenomenon, occurring in about 10% of women taking such agents (personal observations). In addition, NSAIDs may inhibit the motility of the fallopian tubes and by extension, the passage of the oocyte down the tubes [111, 112]. Although these issues might arise with the use of any p0315

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NSAID due to the inhibition of cyclooxygenase, the most widely studied agent is indomethacin [113, 114].

p0320 During pregnancy, naproxen and ibuprofen are the two most commonly used NSAIDs. Indeed, there is too little in the literature regarding any other drug in this class other than the contraindicated indomethacin to have any knowledge regarding their safety profiles. When considering naproxen and ibuprofen in pregnancy, the fetal risk category is B but is reclassified as C when used in high doses. Peripartum there is concern with respect to the neonate regarding intracranial hemorrhage, premature closure of the ductus arteriosus, and impaired renal function leading to a decrease in amniotic fluid volume. The patient should be informed that NSAIDs will start being tapered by week 25 and completely discontinued by week 32 at the latest (6–8 weeks prior to the expected date of delivery).

p0325 A recent population-based study in Denmark indicated that there might be some association with the use of NSAIDs and early pregnancy loss [115]. However, the study fails to indicate the reason for the use of the drugs or if there was an underlying disorder that might predispose to pregnancy loss. It is interesting to note that in women with rheumatoid arthritis, in whom the use of NSAIDs would be quite extensive, there does not appear to be any increased incidence of pregnancy loss. Clearly, further studies need to be undertaken to support or refute the Danish observation.

p0330 Aspirin (ASA) has become a commonly used drug in pregnancy [116]. Despite a continuing lack of consensus, it has been variously recommended as a pretreatment for ovulation induction, for the promotion of implantation in *in vitro* fertilization cycles, and for the prevention of pregnancy loss [117–119]. It has been shown to provide moderate but consistent reductions in the relative risk of preeclampsia and preterm delivery [120]. ASA appears to be a safe agent to use in pregnancy with the exception of a reported questionable increased incidence of gastroschisis in the offspring of women taking ASA in the first trimester [121, 122]. Regardless of the very low frequency of this side effect (1/3000 compared to 1/10,000 in the general population), patients should still be counseled regarding the potential association prior to initiating ASA therapy during pregnancy. ASA is also capable of prolonging labor and may cause an increase in antepartum and postpartum bleeding. It is actually for the latter reasons that it has an FDA classification of C/D when used in the third trimester. To avoid the issues surrounding labor and delivery, discontinuation of ASA 4 weeks prior to the expected date of delivery should be implemented.

p0335 The American Academy of Pediatrics (AAP) considers ASA, naproxen, and ibuprofen compatible with breast-feeding but as always, the lowest effective dose should be used.

Anticoagulants s0165

p0340 The patient with APS and previous thrombosis should receive anticoagulation treatment throughout their pregnancy and during the postpartum period [123]. Data supporting the use of heparin treatment in women with pregnancy loss and APL with no history of thrombosis are inconclusive and evidence-based standards of care both during the peripartum and postpartum periods are lacking [124]. Once the decision to use heparin has been made, low-molecular-weight heparin (LMWH) is perceived to be more desirable than unfractionated heparin (UFH), despite a Cochrane Review recommending the use of unfractionated heparin [125]. LMWH carries less risk of osteoporosis and thrombocytopenia and can be administered once daily [126]. Two small studies comparing UFH and LMWH did not show differences in efficacy but larger prospective studies are needed [127, 128]. LMWH treatment over the duration of a pregnancy can be associated with osteopenia and therefore calcium and vitamin D supplementation should be recommended during pregnancy [129, 130].

Antimalarial Agents s0170

p0345 Antimalarials are used extensively in the treatment of SLE. The major side effect is retinal toxicity, which requires ophthalmologic monitoring every 6–12 months. The favored antimalarial is hydroxychloroquine (FDA Category C), which appears to have a lower incidence of retinal toxicity.

p0350 Numerous studies have attested to the safety of antimalarials in pregnancy [131–133]. All of these investigators have commented that the risk of a flare of disease far outweighs any risk of fetal toxicity. In a follow-up study of the children born to mothers on hydroxychloroquine during pregnancy, Klinger *et al.* found no evidence of retinal toxicity, prompting these authors to conclude that there appears to be little or no risk of ocular toxicity in children exposed to hydroxychloroquine *in utero* [134]. This observation has been confirmed by Motta *et al.* [135].

p0355 Although hydroxychloroquine is eliminated slowly and theoretically could accumulate in neonatal tissues, only 2% of the maternal dose can be detected in breast milk [131]. The AAP have therefore determined that hydroxychloroquine is compatible with breast-feeding.

Corticosteroids s0175

p0360 Corticosteroids are commonly used in the treatment of most rheumatic diseases and are associated with a rapid or relatively rapid therapeutic response. In SLE, prednisone doses in treating minor organ disease

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range from 10 to 40 mg/day and major organ manifestations may be treated with 40–80 mg/day. In all cases, the lowest effective dose of prednisone should be used.

p0365 The use of prednisone in pregnancy is associated with few adverse side effects on the fetus. Maternal side effects are dose-related. The commonest side effects are hypertension and gestational diabetes mellitus. In a double-blind, randomized controlled trial, Laskin *et al.* observed an incidence of gestational diabetes mellitus of 15% and hypertension 13% in the prednisone-treated group compared to 5% in the placebo group for either condition [92]. The cushingoid side effects and osteopenia occur similarly to that seen in the nonpregnant state, the latter necessitating appropriate supplementation with calcium and vitamin D.

p0370 Fetal side effects are few and uncommon. Orofacial clefting in the offspring of mothers treated with corticosteroids during the first trimester has been reported [136, 137]. The results in these studies have been supported by the findings in a recent meta-analysis where the prevalence of orofacial clefting in prednisone-exposed infants was 1 in 400 compared to 1 in 800 in the general population [138]. In spite of the low risk of this potential side effect, any pregnant woman on corticosteroids should be counseled appropriately.

p0375 Premature birth has been described in pregnant women treated with corticosteroids. In a randomized trial referred to above, Laskin *et al.* found premature births before 37 weeks gestation in 62% of the prednisone-treated group compared to 11% in the placebo group [92]. The neonates were all appropriate size for gestational age. Prednisone is FDA classified as D when used in the first trimester. The physician must weigh potential risks vs. benefits when prescribing these agents.

p0380 The AAP considers prednisone to be compatible with breast-feeding. There appears to be minimal exposure with maternal doses at 30–40 mg/day.

s0180 Azathioprine

p0385 Azathioprine (AZA) is used in many rheumatic diseases for its immunosuppressive properties and as a steroid-sparing agent. Among all immunosuppressive agents, AZA appears to be the safest in pregnancy. The placenta reportedly forms a relative barrier to AZA and its metabolites [139]. In a recent study of 189 women exposed to AZA compared to 230 controls not exposed to any teratogens during pregnancy, outcomes associated with AZA included increased rates of spontaneous abortions, intrauterine growth restriction (IUGR), and prematurity [140], but no increase in the occurrence of major malformations. However, as larger studies are required to confirm these results, AZA continues to be a Class D drug.

p0390 With few data available regarding the safety of AZA in breast-feeding, the AAP has recommended that mothers avoid nursing while being treated with this agent.

Methotrexate s0185

p0395 Methotrexate (MTX), a folic acid antagonist, has been used with good success in rheumatoid arthritis and is occasionally used in the treatment of SLE. Use in pregnancy has been associated with spontaneous abortions due to embryotoxicity. The drug has definite association with numerous fetal anomalies as well as IUGR [141–143]. MTX is not to be used in pregnancy and has an FDA Category X rating.

p0400 Owing to MTX binding to tissues, it is recommended that it be discontinued at least 3 months prior to conception. A similar recommendation applies to men taking MTX, but evidence is lacking to support such a recommendation [142]. However, until the situation is clarified, it is recommended that both men and women avoid pregnancy for at least 3 months after discontinuing MTX [141–143].

p0405 MTX is only excreted in breast milk to a very small degree. However, it binds to neonatal tissues and therefore accumulates, leading to toxicity [143]. The AAP categorizes MTX as contraindicated in nursing mothers.

Cyclophosphamide s0190

p0410 Cyclophosphamide (CTX) is a cytotoxic, alkylating agent used in the treatment of severe major organ involvement in SLE. The FDA has categorized it as a Class D drug. CTX is embryotoxic and associated with many anomalies upon exposure in the first trimester. However, it does not appear to be associated with abnormalities if used in the second and third trimesters. Regardless, the drug should not be used in pregnancy unless there is a life-threatening problem and even then restricted to use late in the pregnancy. CTX is contraindicated in the nursing mother owing to the risk of neutropenia, immunosuppression, growth disturbances, and potential carcinogenesis in the neonate [143–145].

Cyclosporine s0195

p0415 Cyclosporine A (CSA, FDA Classification C) is used to treat certain manifestations of SLE renal disease. Most of the literature surrounding the use of CSA in pregnancy deals with renal transplantation. There appears to be little evidence that CSA crosses the placenta significantly [146–148] and current data indicate that it is probably safe in pregnancy with no specific anomalies described [149–151]. Adverse pregnancy outcomes such as prematurity and IUGR likely have

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more to do with the underlying disease process than treatment with CSA [152].

p0420 CSA is excreted in breast milk and is associated with immunosuppression, neutropenia, and growth disturbances in the neonate. The AAP categorizes CSA as contraindicated in the nursing mother.

s0200 Mycophenolate Mofetil

p0425 Mycophenolate mofetil (MMF) is a purine biosynthesis inhibitor. Its use in pregnancy is accompanied by several congenital anomalies and spontaneous abortions [149, 153–157]. These findings have been noted not only in animal studies but also in humans. In addition, a possible characteristic phenotype has been described [154, 158]. Although MMF does not appear to impact male fertility, paternal exposure may be associated with congenital anomalies [151]. Recommendations in women regarding discontinuation of MMF prior to conception vary from 3 to 12 weeks. It would appear that avoidance of this drug by females at least 6 weeks prior to conceiving is most appropriate, whereas for males, the recommendation is 12 weeks' avoidance. This is an FDA Class D drug and should be avoided in pregnancy.

p0430 Since MMF is excreted into breast milk, it should not be administered to nursing mothers.

s0205 Angiotensin-Converting Enzyme Inhibitors (ACE) and Angiotensin Receptor Blockers (ARB)

p0435 ACE inhibitors and ARBs are commonly used in patients with SLE. Their use in pregnancy, however, is contraindicated owing to adverse effects on the fetus. Most of the studies have been performed on patients exposed to ACE inhibitors during pregnancy, but the concerns equally apply to the use of ARBs [159, 160].

p0440 Angiotensin II appears to play a major role in the regulation of uteroplacental blood flow as well as fetal growth, with particular emphasis on the growth of the fetal kidney [159]. Thus use of these antihypertensives may adversely affect maternal blood flow and fetal development. It is necessary to avoid the use of ACE inhibitors and ARBs even in the first trimester due to the risk of cardiovascular and CNS anomalies [160]. Exposure in the second and third trimesters can result in severe fetal adverse effects owing to the effect on fetal renal hemodynamics [161, 162]. The fetal circulation is dependent upon high circulating levels of angiotensin II, which is necessary to maintain the GFR in the normally low-pressure fetal circulation [163]. Use of these agents at this time results in a rapid fall in angiotensin II levels with subsequent fall in GFR, which in turn leads to a decrease in fetal urine production, resulting in a decrease in amniotic fluid volume. Low amniotic fluid volume can then result in developmental abnormalities such as limb contractures, lung hypoplasia, and craniofacial deformities owing to abnormal cranial ossification [161].

p0445 Although some reports indicate recovery of renal function after birth, some renal insufficiency may remain [164]. Therefore, ACE inhibitors and ARBs should be avoided in any woman contemplating a pregnancy and during pregnancy. Alternatives should be prescribed and their use stabilized prior to conception. In addition, those women taking an ACE inhibitor or ARB for the treatment of proteinuria should be assessed after discontinuing the agent to determine if the return of proteinuria now contraindicates pregnancy.

p0450 ACE inhibitors and ARBs are found in breast milk in very low levels. However, owing to the extreme sensitivity of the neonatal kidney to ACE inhibitors in the first few weeks of life, nursing while taking these agents should be avoided until approximately 6 weeks after birth [165].

FAMILY PLANNING AND COUNSELING s0210

p0455 As noted above, a prepregnancy evaluation should be undertaken whenever possible. Apart from the medical assessment, this visit represents the most appropriate time to discuss planning a pregnancy, thereby maximizing the safety of the woman and gaining the highest probability for the desired obstetrical outcome. Many studies have consistently demonstrated that planned pregnancies have better outcomes than those that are unplanned [166]. This is especially the case in those women with an underlying medical problem. Optimizing the health of the prospective mother will improve pregnancy outcome. In the case of the woman with SLE, this will not only involve the assessment of disease activity, but address medication issues. Based upon the findings at this visit, a recommendation for timing a pregnancy will be presented. If there are any medication issues, then substituting a pregnancy-safe medication should be undertaken immediately with plans for follow-up over an appropriate time to ensure that the disease is adequately treated with the newly prescribed alternative medication.

p0460 The mother with lupus contemplating a pregnancy must be counseled not only with respect to her disease entity and the interaction of lupus with pregnancy, but a detailed risk assessment includes a discussion of issues relevant to any woman planning a pregnancy. The counseling session should involve a discussion of maternal age factors and the impact of advanced maternal age

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on conception and pregnancy; weight and nutrition; reproductive and family histories; substance use including tobacco, caffeine, and alcohol; environmental exposures; and psychosocial issues. The clinician must ensure that the patient's partner is enrolled in the decision regarding pregnancy and is fully cognizant of any risks to proceeding.

p0465 The decision to enter into a pregnancy is one that is quite personal and emotional. The prepregnancy evaluation visit provides a unique opportunity for the clinician to establish a trusting relationship with the patient to maximize the probability that she will undertake a pregnancy informed to ensure her safety and that of her future child.

s0215 SLE AND FERTILITY

p0470 Fertility in women with SLE has been reported as normal with pregnancy rates of 2–2.5 observed even in the presence of active disease [160, 167]. Although such studies indicate no difference in fertility rates in women with SLE compared to the general population, one must interpret these findings with caution [168]. Any active inflammatory disorder will impact the pituitary–ovarian axis leading to anovulation. In addition, the adverse effects of certain medications such as cyclophosphamide may severely compromise gonadal function. Therefore, under these circumstances, fertility rates will be affected.

p0475 There are women with well-controlled SLE who may require fertility treatment often in the form of ovulation induction therapy. This treatment requires the use of high doses of exogenous follicle stimulating hormone (FSH), leading to a hyperestrogenic state. This transient hyperestrogenicity may exacerbate lupus. There are several studies of ovulation induction therapy in women with SLE [169–172]. For the most part, the women have fared well, although some complications associated with disease exacerbation have occurred. Since the studies are small, no conclusions can be drawn. Although fertility therapy is not contraindicated in women with SLE, it is advisable to initiate ovulation induction or superovulation only in those individuals whose disease is under excellent control for an extended period such as 6 months. In addition, the patient must be assessed for disease activity and advisability of entering into a pregnancy.

s0220 CONTRACEPTION

p0480 At one time, combined oral contraceptives (estrogen-containing agents) were considered to be at least relatively contraindicated in women with SLE [173–175]. This may still be the case in those with aPL as there appears to be increased risk in those patients of thromboembolism [176]. However, in those lacking aPL the controversies have been limited to patients with some degree of disease activity. Two recent studies clarified this issue. Neither found any association between combined oral contraceptive use and exacerbation of SLE, providing the woman has stable disease [177, 178].

CONCLUSION s0225

p0485 The pregnant woman with SLE represents a unique and potentially complicated management problem for both the rheumatologist/internist and obstetrician/perinatologist. The multisystem nature of the disease and the impact of pregnancy on compromised organ function will challenge the skills of all clinicians involved with the patient. The attending rheumatologist must have detailed knowledge of the clinical disease entity as well as the specific details of the patient's disease history including characteristic clinical manifestations, laboratory markers, and indicators of disease flare. The clinician must know if the patient demonstrates concordancy or discordancy between clinical disease activity and laboratory indicators. Under ideal circumstances, the pregnancy should be planned with the patient and her partner. The woman should be counseled to undertake a pregnancy when the disease is quiescent, after withdrawal of any fetotoxic drugs, and stabilization on medications known to be safe in pregnancy. With improvements in disease management and perinatal monitoring, in addition to collaboration between rheumatologists and perinatologists, there is now a good prognosis for both mother and fetus for the majority of women with systemic lupus erythematosus.

References

[1] A. Doria, L. Iaccarino, S. Arienti, et al., Th2 immune deviation induced by pregnancy: the two faces of autoimmune rheumatic diseases, Reprod Toxicol 22 (2006) 234–241.
[2] C.A. Laskin, Pregnancy and the rheumatic diseases, in: G.N. Burrow, T.P. Duffy, J.A. Copel (Eds.), Medical Complications During Pregnancy, sixth ed., Elsevier Saunders, Philadelphia, PA, 2004, pp. 429–449.
[3] D.W. Branch, Physiologic adaptations of pregnancy, Am. J. Reprod. Immunol. 28 (1992) 120–122.
[4] S.L. Clark, D.B. Cotton, W. Lee, et al., Central hemodynamic assessment of normal term pregnancy, Am J Obstet Gynecol 161 (1989) 1439–1442.
[5] C.J. Lund, J.C. Donovan, Blood volume during pregnancy. Significance of plasma and red cell volumes, Am J Obstet Gynecol 98 (1967) 394–403.
[6] J.A. Pritchard, Changes in blood volume during pregnancy and delivery, Anesthesiology 26 (1965) 393–399.

10035-LAHITA-9780123749949

[7] K. Ueland, Maternal cardiovascular dynamics. VII. Intrapartum blood volume changes, Am J Obstet Gynecol 126 (1976) 671–677.
[8] J.H. Matthews, S. Benjamin, D.S. Gill, et al., Pregnancy associated thrombocytopenia: Definition, incidence and natural history, Acta Haematol 84 (1990) 24–29.
[9] S.F. Kuvin, G. Brecher, Differential neutrophil counts in pregnancy, N Engl J Med 266 (1962) 877–878.
[10] M.J. Paidas, D.H. Ku, Y.S. Arkel, Screening and management of inherited thrombophilias in the setting of adverse pregnancy outcome, Clin Perinatol 31 (2004) 783–805.
[11] P. Lindqvist, B. Dahlback, K. Marsal, Thrombotic risk during pregnancy: a population study, Obstet Gynecol 94 (1999) 595–599.
[12] M. Hellgren, M. Blomback, Studies on blood coagulation and fibrinolysis in pregnancy, during delivery and in the puerperium, Gynecol Obstet Invest 12 (1981) 141–154.
[13] Y. Stirling, L. Woolf, W.R. North, et al., Haemostasis in normal pregnancy, Thromb Haemost 52 (1984) 176–182.
[14] K.A. Bremme, Haemostatic changes in pregnancy, Best Pract Res Clin Haematol 16 (2003) 153–168.
[15] J.A. Hill, Cytokines considered critical in pregnancy, Am J Reprod Immunol 28 (1992) 123.
[16] R.G. Lahita, The effects of sex hormones on the immune system in pregnancy, Am J Reprod Immunol 28 (1992) 136–137.
[17] Z.M. Sthoeger, N. Chiroazzi, R.G. Lahita, Regulation of the immune response by sex hormones. 1. In vivo effects of estradiol and testosterone on poekeweed mitogen induced B-cell differentiation, J Immunol 141 (1988) 91–98.
[18] I.M. Roitt, P.J. Delves, Roitt's Essential Immunology, tenth ed., Blackwell Science, London, 2001.
[19] J.C. Kingdom, P. Kaufmann, Oxygen and placental villous development: origins of fetal hypoxia, Placenta 18 (8) (1997) 613–621.
[20] S. Viero, V. Chaddha, F. Alkazaleh, et al., Prognostic value of placental ultrasound in pregnancies complicated by absent end diastolic flow velocity in umbilical arteries, Placenta 25 (2004) 735–741.
[21] H. Tuchman-Duplessis, G. David, P. Haegel, "Illustrated Human Embryology", vol. I, Springer Verlag, New York, 1975. 62–84.
[22] F.A. Ellis, E.S. Bereston, Lupus erythematosus associated with pregnancy and menopause, AMA Arch Dermatol Syphilol 69 (1952) 170–176.
[23] M. Garsenstein, V.E. Pollak, R.M. Kark, Systemic lupus erythematosus and pregnancy, New England Journal of Medicine 267 (1962) 165–169.
[24] A. Mund, J. Simson, N. Rothfield, Effect of pregnancy on the course of systemic lupus erythematosus, JAMA 183 (1963) 917–920.
[25] R.B. Zurier, Systemic lupus erythematosus and pregnancy, Clinics in Rheumatic Diseases 1 (1975) 613–616.
[26] R.R. Grigor, P.C. Shervington, G.R.V. Hughes, D.F. Hawkins, Outcome of pregnancy in systemic lupus erythematosus. Proc R Soc Med 70: 99–100.
[27] E.C. Tozman, M.B. Urowitz, D.D. Gladman, Systemic lupus erythematosus and pregnancy, J Rheumatol 7 (1980) 624–632.
[28] M.I. Zulman, N. Talal, G.S. Hoffman, W.V. Epstein, Problems associated with the management of pregnancies in patients with systemic lupus erythematosus, J Rheumatol 7 (1980) 37–49.
[29] M.D. Lockshin, E. Reinitz, M.L. Druzin, M. Murrman, D. Estes, Lupus pregnancy: case control study demonstrating absence of lupus exacerbations during and after pregnancy, Am J Med 77 (1984) 893–898.
[30] M.D. Lockshin, Pregnancy does not cause systemic lupus erythematosus to worsen, Arthritis Rheum 32 (1989) 665–670.
[31] M. Petri, Hopkins lupus pregnancy center: 1987-1996, Rheum Dis Clin North Am 23 (1997) 1–13.
[32] G. Ruiz-Irastortza, F. Lima, J. Alves, et al., Increased rate of lupus flare during pregnancy and the puerperium: a prospective study of 78 pregnancies, Br J Rheumatol 35 (1996) 133–138.
[33] J. Cortes-Hernandez, J. Ordi-Ros, F. Paredes, M. Casellas, F. Castillo, M. Vilardell-Tarres, Clinical predictors of fetal and maternal outcome in systemic lupus erythematosus: a prospective stud of 103 pregnancies, Rheumatology (Oxford) 41 (2002) 643–650.
[34] M.B. Urowitz, D.D. Gladman, V.T. Farewell, J. Stewart, J. McDonald, Lupus and pregnancy studies, Arthritis Rheum 36 (1993) 1392–1397.
[35] J.P. Buyon, K.C. Kalunian, R. Ramsey-Goldman, M.A. Petri, G. Ruiz-Irastorza, M. Khamashta, Assessing disease activity in SLE patients during pregnancy, Lupus 8 (1999) 677–684.
[36] G. Ruiz-Irastorza, M.A. Khamashta, Evaluation of systemic lupus erythematosus activity during pregnancy, Lupus 13 (2004) 679–682.
[37] S. Yasmeen, E.E. Wilkins, N.T. Field, R.A. Sheikh, W.M. Gilbert, Pregnancy outcomes in women with systemic lupus erythematosus, J Maternal Fetal Med 10 (2001) 91–96.
[38] C.A. Clark, K.A. Spitzer, J.N. Nadler, C.A. Laskin, Preterm deliveries in women with systemic lupus erythematosus, J Rheumatol 30 (2003) 2127–2132.
[39] J.P. Hayslett, Kidney disease and pregnancy, Kidney Int 25 (1984) 579–587.
[40] M. Surian, E. Imbasciati, P. Cosci, Glomerular disease and pregnancy: A study of 123 pregnancies inpatients with primary and secondary glomerular diseases, Nephron 36 (1984) 101–105.
[41] P. Jungers, P. Houillier, D. Forget, et al., Influence of pregnancy on the course of primary chronic glomerulonephritis, Lancet 346 (1995) 1122–1224.
[42] S. Hou, Pregnancy in women with chronic renal disease, N Engl J Med 312 (1985) 836–839.
[43] E. Imbasciati, G. Pardi, P. Capetta, et al., Pregnancy in women with chronic renal failure, Am J Nephrol 6 (1986) 193–198.
[44] E. Imbasciati, G. Gregorini, G. Cabiddu, et al., Pregnancy in CKD stages 3 to5: Fetal and maternal outcomes, Am J Kidney Dis 49 (2007) 753–762.
[45] P. Jungers, P. Houillier, D. Forget, M. Henry-Amar, Specific controversies concerning the natural history of renal disease in pregnancy, Am J Kidney Dis 17 (1991) 116–122.
[46] P. Jungers, M. Dougados, C. Pelissier, et al., Lupus nephropathy and pregnancy, Arch Intern Med 142 (1982) 771–776.
[47] D.L. Huong, B. Wechsler, D. Vauthier-Brouzes, et al., Pregnancy in past or present lupus nephritis: a study of 32 pregnancies from a single centre, Ann Rheum Dis 60 (2001) 599–604.
[48] G. Bobrie, F. Liote, P. Houillier, et al., Pregnancy in lupus nephritis and related disorders, Am J Kidney Dis 9 (1987) 339–343.
[49] G. Moroni, S. Quaglini, G. Banfi, et al., Pregnancy in lupus nephritis, Am J Kidney Dis 40 (2002) 713–720.
[50] A. Tandon, D. Ibaniz, D.D. Gladman, M.B. Urowitz, The effect of pregnancy on lupus nephritis, Arthritis Rheum 50 (2004) 3941–3946.
[51] ACOG Committee on Practice Bulletins – Obstetrics, ACOG practice bulletin. Diagnosis and management of preeclampsia and eclampsia, Obstet Gynecol 99 (2002) 159–167.
[52] K.Y. Lain, J.M. Roberts, Contemporary concepts of the pathogenesis and management of preeclampsia, JAMA 287 (2002) 3183–3186.

10035-LAHITA-9780123749949

[53] M.D. Lockshin, Pregnancy does not cause SLE to worsen, Arthritis Rheum 32 (1989) 665-370.
[54] H.C. Nossent, T.J. Swaak, Systemic lupus erythematosus. VI. Analysis of the interrelationship with pregnancy, J Rheumatol 17 (1990) 771–776.
[55] G. Ruiz-Irastorza, M.A. Khamashta, Lupus and pregnancy: ten questions and some answers, Lupus 17 (2008) 416–420.
[56] J.H. Matthews, S. Benjamin, D.S. Gill, et al., Pregnancy-associated thrombocytopenia: Definition, incidence and natural history, Acta Haematol 84 (1990) 24–29.
[57] V. Fenton, K. Saunders, I. Cavill, The platelet count in pregnancy, J Clin Pathol 30 (1977) 68–69.
[58] P.R. Sill, T. Lind, W. Walker, Platelet values during normal pregnancy, Br J Obstet Gynaecol 92 (1985) 480–483.
[59] A.S. Sejeny, R.D. Eastham, S.R. Baker, Platelet counts during normal pregnancy, J Clin Pathol 28 (1975) 812–813.
[60] R.F. Burrows, J.G. Kelton, Fetal thrombocytopenia and its relation to maternal thrombocytopenia, N Engl J Med 329 (1993) 1463–1466.
[61] J.N. George, S.H. Woolf, G.E. Raskob, et al., Idiopathic thrombocytopenic purpura. A practice guideline developed by explicit methods for the America Society of Hematology, Blood 88 (1996) 3–40.
[62] R.F. Burrows, J.G. Kelton, Platelets and pregnancy, in: R.V. Lee (Ed.), Current Obstetric Medicine, vol. 2, Mosby-Year Book, St. Louis, 1993, pp. 83–106.
[63] D. Alarcon-Segovia, J. Sanchez-Guerrero, Correction of thrombocytopenia with small dose aspirin in the primary antiphospholipid antibody syndrome, J. Rheumatol. 16 (1989) 1359–1361.
[64] S.D. Payne, R. Resnik, T.R. Moore, et al., Maternal characteristics and risk of severe neonatal thrombocytopenia and intracranial hemorrhage in pregnancies complicated by autoimmune thrombocytopenia, Am J Obstet Gyencol 177 (1997) 149–155.
[65] A.S. Valat, M.T. Caulier, P. Devos, et al., Relationships between severe neonatal thrombocytopenia and maternal characteristics in pregnancies associated with autoimmune thrombocytopenia, Br J Haematol 103 (1998) 397–401.
[66] K. Fujimura, Y. Harada, T. Fujimoto, et al., Nationwide study of idiopathic thrombocytopenic purpura in pregnant women and the clinical influence on neonates, Int J Hematol 75 (2002) 426–433.
[67] J.O. Martinez-Rueda, C.A. Arce-Salinas, A. Kraus, et al., Factors associated with fetal losses in severe systemic lupus erythematosus, Lupus 5 (1996) 113–119.
[68] C.S. Kuzis, M.L. Druzin, R.E. Lambert, Case report: A patient with severe CNS lupus during pregnancy, Ann. Med. Intern. 147 (1996) 274–275.
[69] R.E. Wolf, J.G. McBeath, Chorea gravidarum in systemic lupus erythematosus, J. Rheumatol. 12 (1985) 992–993.
[70] M. Marabani, A. Zoma, D. Hadley, et al., Transverse myelitis occurring during pregnancy in a patient with systemic lupus erythematosus, Ann. Rheum. Dis. 48 (1989) 160–162.
[71] W.I. Morris, Pregnancy in rheumatoid arthritis and systemic lupus erythematosus, Aust N Z J Obstet Gynecol 9 (1969) 136–144.
[72] E.A. Friedman, J.W. Rutherford, Pregnancy and lupus erythematosus, Obstet Gynecol 8 (1956) 601–610.
[73] F. Lima, N.M.M. Buchanan, M.A. Kamashta, S. Kerslake, G.R. Hughes, Obstetric outcome in systemic lupus erythematosus, Semin Arthritis Rheum 25 (1995) 184–192.
[74] C.A. Clark, K.A. Spitzer, C.A. Laskin, Decrease in pregnancy loss rates in patients with systemic lupus erythematosus over a 40-year period, J Rheumatol 32 (2005) 1709–1712.
[75] H.P. McNeill, C.N. Chesterman, S.A. Krilis, Immunology and clinical importance of antiphospholipid antibodies, Adv Immunol 8 (1991) 425–426.
[76] C.E. Tseng, J.P. Buyon, Neonatal lupus syndrome, Rheumatic Dis Clin North Am 23 (1997) 31–54.
[77] M. Elias, A. Eldor, Thromboembolism in patients with the "lupus" type circulating anticoagulant, Arch Intern Med 144 (1984) 510–515.
[78] J.R. Mueh, K.D. Herbst, S.I. Rapaport, Thrombosis in patients with the lupus anticoagulant, Ann Intern Med 92 (1980) 156–159.
[79] M.D. Lockshin, M.L. Druzin, S. Goei, et al., Antibody to cardiolipin as a predictor of fetal distress or death in pregnant patients with systemic lupus erythematosus, N Engl J Med 313 (1985) 152–156.
[80] C.A. Clark-Soloninka, K.A. Spitzer, J.N. Nadler, C.A. Laskin, Evaluation of screening 590 plasma samples for the lupus anticoagulant using a panel of four tests, Arthritis Rheum 41 (Suppl.) (1998) S168.
[81] J.E. Salmon, M. Kim, M. Guerra, et al., Absence of lupus anticoagulant is a strong predictor of uncomplicated pregnancy in patients with APL antibodies, Arthritis Rheum 58 (Suppl.) (2008) S403.
[82] J.O. Martinez-Rueda, C.A. Arce-Salinas, A. Kraus, J. Alcocer-Varela, D. Alarcon-Segovia, Factors associated with fetal loss in severe systemic lupus erythematosus, Lupus 5 (1996) 113–119.
[83] J.G. Hanly, D.D. Gladman, T.H. Rose, C.A. Laskin, M.B. Urowitz, Lupus pregnancy: A prospective study of placental changes, Arthritis Rheum 31 (1998) 358–366.
[84] C.R. Abramowsky, M.E. Vegas, G. Swinehart, M.T. Gyves, Decidual vasculopathy of the placenta in lupus erythematosus, N Engl J Med 303 (1980) 668–672.
[85] E. Guzman, H. Schulman, L. Bracero, B. Rochelson, G. Farmakides, A. Coury, Uterine-umbilical artery Doppler velocimetry in pregnant women with systemic lupus erythematosus, J Ultrasound Med 11 (1992) 275–281.
[86] L.O. Carreras, G. Defreyn, S.J. Machin, et al., Arterial thrombosis, intrauterine death and "lupus" anticoagulant: Detection of immunoglobulin interfering with prostacyclin formation, Lancet 1 (1981) 244–246.
[87] W. Geis, D.W. Branch, Obstetric implications of antiphospholipid antibodies: pregnancy loss and other complications, Clin Obstet Gynecol 44 (2001) 2–10.
[88] H.J. Out, H.W. Bruinsem, G.C.M.L. Chrustuaebs, et al., A prospective, controlled multicenter study on the obstetric risks of pregnant women with antiphospholipid antibodies, Am J Obstet Gynecol 167 (1992) 26–32.
[89] D. Vinatier, P. Dufour, M. Cosson, J.L. Houpeau, Antiphospholipid syndrome and recurrent miscarriages, Eur J Obstet Gynecol Reprod Biol 96 (2001) 37–50.
[90] C.A. Soloninka, C.A. Laskin, J. Wither, D. Wong, C. Bombardier, J. Raboud, Clinical utility and specificity of anticardiolipin antibodies, J Rheumatol 18 (1991) 1849–1855.
[91] W.F.F. Lubbe, W.S. Butler, S.J. Palmer, G.C. Liggins, Fetal survival after prednisone suppression of maternal lupus anticoagulant, Lancet 2 (1983) 1361–1363.
[92] C.A. Laskin, C. Bombardier, M. Hannah, et al., Prednisone and aspirin in women with autoantibodies and unexplained recurrent fetal loss, N Engl J Med 337 (1997) 148–153.
[93] R. Rai, H. Cohen, M. Dave, L. Regan, Randomized controlled trial of aspirin and aspirin plus heparin in pregnant women with recurrent miscarriage associated with phospholipids antibodies (or antiphospholipid antibodies), Br Med J 314 (1997) 253–257.

10035-LAHITA-9780123749949

[94] D.L. Huong, B. Wechsler, O. Bletry, D. Vauthier-Brouzes, G. Lefebvre, J.C. Piette, A study of 75 pregnancies in patients with antiphospholipid syndrome, J Rheumatol 28 (2001) 2025–2030.
[95] R.G. Farquharson, S. Quenby, M. Greaves, Antiphospholipid syndrome in pregnancy: a randomized, controlled trial of treatment, Obstet Gynecol 100 (2002) 408–413.
[96] H.A. Shehata, C. Nelson-Piercy, M.A. Khamashta, Management of pregnancy in antiphospholipid syndrome, Rheum Dis Clin North Am 27 (2001) 643–659.
[97] C.A. Laskin, J. Ginsberg, M. Crowther, et al., Results from the HepASA trial: low molecular weight heparin + ASA vs ASA only for recurrent pregnancy loss, Arthritis Rheum 54 (Suppl.) (2006) S558.
[98] B. Bresnihan, R.R. Grigor, M. Oliver, et al., Immunological mechanism for spontaneous abortion in systemic lupus erythematosus, Lancet 2 (1997) 1205–1207.
[99] M. Empson, M. Lassere, J.C. Craig, J.R. Scott, Recurrent pregnancy loss with antiphospholipid antibody: a systematic review of therapeutic trials, Obstet Gynecol 100 (2002) 135–144.
[100] F. Meccacci, B. Bianchi, A. Peiralll, et al., Pregnancy outcome in systemic lupus erythematosus complicated by anti-phospholipid antibodies, Rheumatology (Oxford) 48 (2009) 246–249.
[101] D.M. Freidman, M.Y. Kim, J.A. Copel, et al., Utility of cardiac monitoring in fetuses at risk for congenital heart block: the PR Interval and Dexamethasone Evaluation (PRIDE) prospective study, Circulation 117 (2008) 485–493.
[102] A. Brucato, A. Doria, M. Frassi, et al., Pregnancy outcome in 100 women with autoimmune diseases and anti-Ro/SSA antibodies: a prospective controlled study, Lupus 11 (2002) 716–721.
[103] C. Llanos, P.M. Izmirly, M. Katholi, et al., Recurrence rates of cardiac manifestations associated with neonatal lupus and maternal/fetal risk factors, Arthritis Rheum 60 (2009) 3091–3097.
[104] J.Y. Lee, P.T. Huerta, J. Zhang, C. Kowal, E. Bertini, B.T. Volpe, et al., Maternal lupus and congenital cortical impairment, Nat Med 15 (2009) 91–96.
[105] J. Sanchez-Guerrero, C. Aranow, M. Mackay, B. Volpe, B. Diamond, Neuropsychiatric systemic lupus erythematosus reconsidered, Nat Clin Pract Rheumatol 4 (2009) 112–113.
[106] G. Ruiz-Irastorza, F. Lima, J. Alvers, et al., Increased rate of lupus flare during pregnancy and the puerperium: a prospective study of 78 pregnancies, Br J Rheumatol 35 (1996) 133–138.
[107] M.B. Urowitz, D.D. Gladman, Measures of disease activity and damage in SLE, Baillière's Clin Rheumatol 12 (1998) 405–413.
[108] R.M. Andrade, G. McGwin Jr., G.S. Alarcon, et al., Predictors of post-partum damage accrual in systemic lupus erythematosus: data from LUMINA, a multiethnic US cohort (XXXVII), Rheumatology (Oxford) 45 (2006) 1380–1384.
[109] S. Stone, M.A. Khamashta, C. Nelson-Piercy, Nonsteroidal anti-inflammatory drugs and reversible female infertility: is there a link? Drug Safety 25 (2002) 545–551.
[110] S. Killick, M. Elstein, Pharmacologic production of luteinized unruptured follicles by prostaglandin synthetase inhibitors, Fertil Steril 47 (1987) 773–777.
[111] M.G. Elder, L. Myatt, G. Chaudhuri, The role of prostaglandins in the spontaneous motility of lthe fallopian tube, Fertil Steril 28 (1997) 86–90.
[112] A. Laszlo, G.L. Nadasy, E. Monos, B. Zsolnai, Effect of pharmacological agents on the activity of the circular and longitudinal smooth muscle layers of human fallopian tube ampullar segments, Acta Physiol Hungarica 72 (1988) 123–133.
[113] N. Sookvanichsilp, P. Pulbutr, Anti-implantation effects of indomethacin and celecoxib in rats, Contraception 65 (2002) 373–378.
[114] H. Lim, B.C. Paria, S.K. Das, et al., Mulitple female reproductive failures in cyclooxygenase 2-deficient mice, Cell 91 (1997) 197–208.
[115] G.L. Nielsen, H.T. Sorensen, H. Larsen, L. Pedersen, Risk of adverse birth outcome and miscarriage in pregnant users of non-steroidal anti-inflammatory drugs: population based observational study and case-control study, Br Med J 322 (2001) 266–270.
[116] A.H. James, L.R. Brancazio, T. Price, Aspirin and reproductive outcomes, Obstet Gynecol Surv 63 (2008) 49–57.
[117] J.G. Bromer, M.B. Cetinkaya, A. Arici, Tretreatments before the induction of ovulation in assisted reproduction technologies: evidence-based medicine in 2007, Ann N Y Acad Sci 1127 (2008) 31–40.
[118] J.L. Frattarelli, G.D. McWilliams, M.J. Hill, K.A. Miller, R.T. Scott Jr., Low-dose aspirin use does not improve in vitro fertilization outcomes in poor responders, Fertil Steril 89 (2008) 1113–1117.
[119] E. Jauniaux, R.G. Farquharson, O.B. Christiansen, N. Exalto, Evidence-based guidelines for the investigation and medical treatment of recurrent miscarriage, Human Reprod 21 (2006) 2216–2222.
[120] L.M. Askie, L. Duley, D.J. Henderson-Smart, L.A. Stewart, and the PARIS Collaborative Group. Antiplatelet gents for prevention of pre-eclampsia: a meta-analysis of individual patient data, Lancet 369 (2007) 1791–1798.
[121] E. Kozer, S. Nikfar, A. Costei, R. Boskovic, I. Hulman, G. Koren, Aspirin consumption during the first trimester of pregnancy and congenital anomalies: a meta-analysis, Am J Obstet Gynecol 187 (2002) 1623–1630.
[122] B. Norgard, E. Puho, A.E. Czeizel, M.V. Skriver, H.T. Sorensen, Aspirin use during early pregnancy and the risk of congenital abnormalities: a population-based case-control study, Am J Obstet Gynecol 192 (2005) 922–923.
[123] G. Riuz-Irastorza, M.A. Khamashta, Management of thrombosis in antiphospholipid syndrome and systemic lupus erythematosus in pregnancy, Ann N Y Acad Sci 1051 (2005) 606–612.
[124] K.A. Spitzer, D. Murphy, M. Crowther, C.A. Clark, C.A. Laskin, Postpartum management of women at increased risk of thrombosis: results of a Canadian pilot study, J Rheumatol 33 (2006) 2222–2226.
[125] M. Empson, M. Lassere, J. Craig, J. Scott, Prevention of recurrent miscarriage for women with antiphospholipid antibody or lupus anticoagulant, Cochrane Database System Reviews 18 (2005) CDD002859.
[126] I.A. Greer, Anticoagulants in pregnancy, J Thromb Thrombolysis 21 (2006) 57–65.
[127] M.D. Stephenson, P.J. Ballem, P. Tsang, Treatment of antiphospholipid antibody syndrome (APS) in pregnancy: a randomized pilot comparing low molecular weight heparin to unfractionated heparin, Canad J Obstet Gynecol 26 (2004) 729–734.
[128] L.S. Noble, W.H. Kutteh, N. Lashey, R.D. Frandklin, J. Herrad, Antiphospholipid antibodies associated with recurrent pregnancy loss: prospective, multicenter, controlled pilot study comparing treatment with low molecular weight heparin vs unfractionated heparin, Fertil Steril 83 (2005) 684–690.
[129] G. Ruiz-Irastorza, M.A. Khamashta, C. Nelson-Piercy, G.R. Hughes, Lupus pregnancy: is heparin a risk factor for osteoporosis? Lupus 10 (2001) 597–600.

10035-LAHITA-9780123749949

[130] P. Deruelle, C. Coulon, The use of low molecular weight heparins in pregnancy—how safe are they? Curr Opin Obstet Gynecol 19 (2007) 573–577.
[131] A. Al-Herz, M. Schulzer, J.M. Esdaile, Survey of antimalarial use in lupus pregnancy and lactation, J Rheumatol 29 (2002) 700–706.
[132] M.E. Clowse, L. Magder, R. Witter, M. Petri, Hydroxychloroquine in lupus pregnancy, Arthritis Rheum 54 (2006) 3640–3647.
[133] M.A. Khamashta, N.M. Buchanan, G.R. Hughes, The use of hydroxychloroquine in lupus pregnancy: the British experience, Lupus 5 (Suppl. 1) (1996) S65–S66.
[134] G. Klinger, Y. Morad, C.A. Westall, et al., Ocular toxicity and antenatal exposure to chloroquine or hydroxychloroquine for rheumatic diseases, Lancet 358 (2001) 813–814.
[135] M. Motta, A. Tincai, D. Faden, et al., Follow-up of infants exposed to hydroxychloroquine given to mothers during pregnancy and lactation, J Perinatol 25 (2005) 86–89.
[136] S.L. Carmichael, G.M. Shaw, C. Ma, et al., and the National Birth Defects Prevention Study, Maternal corticosteroid use and orofacial cleft, Am J Obstet Gynecol 197 (2007) 585. el-587.
[137] P. Pradat, E. Robert-Gnansia, G.L. Di Tanna, et al., and contributors to the MADRE database, 2003 First trimester exposure to corticosteroids and oral clefts, Birth Defects Res A Clin Mol Teratol 67 (2003) 968–970.
[138] L. Park-Wyllie, P. Mazzotta, A. Pastuszak, et al., Birth defects after maternal exposure to corticosteroids: prospective cohort study and meta analysis of epidemiological studies, Teratology 62 (2000) 385–392.
[139] N.K. de Boer, S.V. Jarbandhan, P. de Graaf, C.J. Mulder, R.M. van Elburg, A.A. van Bodergraven, Azathioprine use during pregnancy: unexpected intrauterine exposure to metabolites, Am J Gastroenterol 101 (2006) 1390–1392.
[140] L.H. Goldstein, G. Dolinsky, R. Greenberg, et al., Birth Defects Res A Clin Mol Teratol 79 (2007) 696–701.
[141] R. Ramsey-Goldman, E. Schilling, Immunosuppressive drug use during pregnancy, Rheum Dis Clin North Am 23 (1997) 149–167.
[142] A.E. French, G. Koren, and the Motherisk Team. Effect of methotrexate on male fertility, Can Fam Physician 49 (2003) 577–578.
[143] K.K. Temprano, R. Branlamudi, T.L. Moore, Antirheumatic drugs in pregnancy and lactation, Semin Arthritis Rheum 35 (2005) 112–121.
[144] N.M. Janssen, M.S. Genta, The effects of immunosuppressive and anti-inflammatory medication on fertility, pregnancy, and lactation, Arch Intern Med 160 (2000) 610–619.
[145] M. Ostensen, Treatment with immunosuppressive and disease modifying drugs during pregnancy and lactation, Am J Reprod Immunol 28 (1992) 148–152.
[146] M. Nandakumaran, A.S. Eldeen, Transfer of cyclosporine in the perfused human placenta, Dev Pharmacol Ther 15 (1990) 101–105.
[147] R. Venkataramanan, B. Koneru, C.C. Wang, G.J. Burckart, S.N. Caritis, T.E. Starzl, Cyclosporine and its metabolites in mother and baby, Transplantation 46 (1988) 468–469.
[148] S. Di Paolo, R. Monno, G. Stallone, et al., Placental imbalance of vasoactive factors does not affect pregnancy outcome in patients treated with Cyclosporine A after transplantation, Am J Kidney Dis 39 (2002) 776–783.
[149] M. Ostensen, M. Khamashta, M. Lockshin, et al., Anti-inflammatory and immunosuppressive drugs and reproduction, Arthritis Res Ther 8 (2006) 209.
[150] I. Cockburn, P. Krupp, C. Monka, Present experience of Sandimmun in pregnancy, Transplant Proc 21 (1989) 3730–3732.
[151] V.T. Armenti, K.M. Ahlswede, B.A. Ahlswede, et al., National Transplantation Pregnancy Registry: Outcomes of 154 pregnancies in cyclosporine-treated female kidney transplant recipients, Transplantation 57 (1994) 502–506.
[152] B. Bar Oz, R. Hackman, T. Einarson, G. Koren, Pregnancy outcome after cyclosporine therapy during pregnancy: a meta-analysis, Transplantation 71 (2001) 1051–1055.
[153] N.M. Sifontis, L.A. Coscia, S. Constaninescu, A.F. Lavelanet, M.J. Moritz, V.T. Armenti, Pregnancy outcomes in solid organ transplant recipients with exposure to mycophenolate mofetil or sirolimus, Transplantation 82 (2006) 1698–1702.
[154] A. Perez-Aytess, A. Ledo, V. Boso, et al., In utero exposure to mycophenolate mofetil: a characteristic phenotype? Am J Med Genet 146 (2008) 1–7.
[155] S.M. Downs, Induction of meiotic maturation in vivo in the mouse by IMP dehydrogenase inhibitors: effects on the developmental capacity of ova, Mol Reprod Develop 38 (1994) 293–302.
[156] M.A. Parisi, H. Zayed, A.M. Slavotinek, J.C. Rutledge, Congential diaphragmatic hernia and microtia in a newborn with mycophenolate mofetil (MMF) exposure: phenocopy for Fryne syndrome or broad spectrum of teratogenic effects? Am J Med Genet A 149A (2009) 1237–1240.
[157] M.T. Anderka, A.E. Lin, D.N. Abuelo, A.A. Mitchell, S.A. Rasmussen, Reviewing the evidence of mycophenolate mofetil as a new teratogen: case report and review of the literature, Am J Med Genet A 149A (2009) 1241–1248.
[158] P. Merlob, B. Stahl, G. Klinger, Tetrada of possible mycophenolate mofetil embyopathy: a review, Reprod Toxicol 28 (2009) 105–108.
[159] Risks of angiotensin-converting enzyme inhibition during pregnancy: experimental and clinical evidence, potential mechanisms, and recommendations for use. Shotan A; Widerhorn J; Hurst A; Elkayam U Am J Med 96 (1994) 451–456.
[160] Major congenital malformations after first-trimester exposure to ACE inhibitors. Cooper WO; Hernandez-Diaz S; Arbogast PG; Dudley JA; Dyer S; Gideon PS; Hall K; Ray WA N Engl J Med 354 (2006) 2443–2451.
[161] Angiotensin-converting enzyme inhibitor fetopathy. Pryde PG; Sedman AB; Nugent CE; Barr M Jr J Am Soc Nephrol 3 (1993) 1575–1582.
[162] Adverse pregnancy outcomes associated with maternal enalapril antihypertensive treatment. Tabacova S; Little R; Tsong Y; Vega A; Kimmel CA Pharmacoepidemiol Drug Saf 12 (2003) 633–646.
[163] An intact renin-angiotensin system is a prerequisite for normal renal development. Guron G; Friberg P J Hypertens 18 (2000) 123–137.
[164] G.D. Simonetti, T. Baumann, J.M. Pachlopnik, et al., Non-lethal fetal toxicity of the angiotensin receptor blocker candesartan, Pediatr Nephrol 21 (2006) 1329.
[165] American Academy of Pediatrics, Committee on Drugs. The transfer of drugs and other chemicals into human milk, Pediatrics 108 (2001) 776–789.
[166] K. Johnson, S.F. Posner, J. Biermann, et al., Recommendations to improve preconception health and health care- United States A report of the CDC/ATSDR Preconception Care Work Group and the Select Panel on Preconception Care, MMWR Recomm Rep 55 (2006) 1.
[167] C.C. Mok, R.W. Wong, Pregnancy in systemic lupus erythematosus, Postgrad Med J 77 (2001) 157–165.
[168] A. Fraga, G. Mintz, J. Orozco, Sterility and fertility rates, fetal wastage and maternal morbidity in systemic lupus erythematosus, J Rheumatol 1 (1974) 283–288.

10035-LAHITA-9780123749949

[169] J. Sanchez-Guerrero, E.W. Karlson, M.H. Liang, D.J. Hunter, F.E. Speizer, G.A. Colditz, Past use of oral contraceptives and the risk of developing systemic lupus erythematosus, Arthritis Rheum 40 (1997) 804–808.
[170] D.L. Huong, B. Wechsler, J.C. Piette, Risks of ovulation induction therapy in systemic lupus erythematosus, Br J Rheumatol 35 (1996) 1184–1186.
[171] D.L. Huong, B. Wechsler, D. Vauthier-Brouzes, et al., Importance of planning ovulation in systemic lupus erythematosus and antiphospholipid syndrome: a single center retrospective study of 21 cases and 114 cycles, Semin Arthritis Rheum 32 (2002) 174–188.
[172] N. Guballa, L. Sammaritano, S. Schwartzman, J. Buyon, M.D. Lockshin, Ovulation induction and in vitro fertilization in systemic lupus erythematosus and antiphospholipid syndrome, Arthritis Rheum 43 (2002) 550–556.
[173] G.S. Cooper, M.A. Dooley, E.L. Treadwell, E.W. St Clair, G.S. Gilkeson, Hormonal and reproductive risk factors for development of systemic lupus erythematosus: results of a population-based, case-control study, Arthritis Rheum 46 (2002) 1830–1839.
[174] D. Gill, Rheumatic complaints of women using antiovulatory drugs, J Chron Dis 21 (1968) 435–444.
[175] E.L. Dubois, L. Strain, M. Ehn, G. Bernstein, G.J. Friou, LE cells after oral contraceptives, Lancet 2 (1968) 679.
[176] T.A. Chapel, R.E. Burns, Oral contraceptives and exacerbation of lupus erythematosus, Am J Obstet Gynecol 110 (1971) 366–369.
[177] M. Petri, M.Y. Kim, K.C. Kalunian, et al., Combined oral contraceptives in women with systemic lupus erythematosus, N Engl J Med 353 (2005) 2550–2558.
[178] J. Sanchez-Guerrero, A.G. Uribe, L. Jimenez-Santana, et al., A trial of contraceptive methods in women with systemic lupus erythematosus, N Engl J Med 353 (2005) 2539–2549.

10035-LAHITA-9780123749949